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Surfacant Contamination Alters Freshwater Phytoplankton Community Composition

William H. Hodge

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SURFACTANT CONTAMINATION ALTERS FRESHWATER PHYTOPLANKTON COMMUNITY COMPOSITION

by

WILLIAM HODGE

(Under the Direction of Risa A. Cohen)

ABSTRACT

Pollution from cleaning and personal care products enter freshwater systems and have the potential to alter phytoplankton abundance and diversity. Alkyl polyglucoside (APG), a widely used foaming agent in detergents, decreases phytoplankton abundance, but whether sensitivity to APG is affected by taxonomic identity and/or the presence of competitors is unclear. Establishing taxon-specific responses to APG is important, because taxa differ in nutritional quality and palatability for zooplankton grazers. Chapter one describes comparisons between how individual phytoplankton communities respond to the same range of APG concentrations to test hypotheses that: 1) chemical concentration determines how individual taxa respond to APG; 2) individual taxon responses to APG concentration are affected by the presence of competitors. Microcosms were inoculated with either individual phytoplankton species or communities with known cell densities and exposed to one of five APG treatments: 0 (control), 0.01, 0.5, 2, or 10 mg L⁻¹. Cell density and chlorophyll-*a* concentration responses were used to determine APG effects on phytoplankton. Results indicated that in the lab, changes in community composition were due to losses of *Microcystis aeruginosa*, while in the field changes were mostly due to losses of *Navicula* sp. However, natural communities are comprised of many more phytoplankton species than constructed communities, and grazers and other trophic levels are present. Chapter two describes a comparison of two experiments, one experiment where ambient phytoplankton communities were exposed to APG with zooplankton grazers, and one experiment without zooplankton grazers. The hypothesis was that APG influences phytoplankton community composition more in the presence of zooplankton grazers.

Results indicate that there was no observed effect of APG without grazers, but with grazers APG influenced community composition and *Chlorella* sp. abundance. The presence of zooplankton resulted in reduced abundance of palatable taxa considered to be high quality food for grazers.

INDEX WORDS: Alkyl polyglucoside, Phytoplankton, Pollutants, Zooplankton

SURFACTANT CONTAMINATION ALTERS FRESHWATER PHYTOPLANKTON
COMMUNITY COMPOSITION

by

WILLIAM HODGE

B.S. Seton Hill University, 2016

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial

Fulfillment of the Requirements for the Degree

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CHAPTER 1

SPECIES IDENTITY AND THE PRESENCE OF COMPETITORS DETERMINE PHYTOPLANKTON RESPONSES TO ALKYL POLYGLUCOSIDE CONTAMINATION

INTRODUCTION

Phytoplankton communities provide food resources to consumers in aquatic ecosystems, but the overall abundance of phytoplankton may not necessarily reflect food availability (Choi et al. 2014, Sarmiento et al. 2016). The taxonomic composition of a phytoplankton community is an important determinant of the amount and quality of food available, at least in part because not all taxa are palatable to or have high nutritional quality for grazers (Porter and Orcutt 1980, Sterner et al. 1993, Brett and Muller-Navarra 1997, Choi et al. 2016). Freshwater phytoplankton communities include a high diversity of taxa, including cyanobacteria (Cyanophyceae), green algae (Chlorophyceae), diatoms (Bacillariophyceae), and euglenoids (Euglenophyceae) (Affan et al. 2005). Unicellular green algae and diatoms rich in fatty acids are often preferentially consumed by grazers, although diatoms low in frustule silica content are also generally preferred (Liu et al. 2016). In contrast, cyanobacteria are typically deficient in lipid content and have filamentous/colonial morphology that makes them difficult for grazers to consume (Porter and Orcutt 1980, Brett and Muller-Navarra 1997). Cyanobacteria may also produce toxic secondary metabolites that contribute to decreased freshwater zooplankton abundance (Wilson et al. 2006, Zhu et al. 2013, Ger et al. 2014, Lyu et al. 2017). Thus it is important to determine which phytoplankton taxa are present in a community to assess food web support to higher trophic levels.

Changes in phytoplankton community composition due to seasonal succession are expected to occur following alterations of nutrient and light availability, temperature, and zooplankton grazing (Ryther and Sanders 1980, Malone and Neale 1981, Sommer et al. 1986, Sommer et al. 2012). For example, in temperate lakes, spring blooms of diatoms and green algae result from increased light and nutrient availability followed by decreases in population size due to increased zooplankton abundance and grazing pressure (Sommer et al. 1986). As temperatures warm and nutrient concentrations decrease in the summer, cyanobacteria (e.g. *Raphidiopsis* spp. and *Clindrospermopsis* spp.) increase in biomass while diatoms and green algae continue to decrease (Fonseca and Bicudo 2008). When temperatures cool in the fall, cyanobacteria are typically replaced by diatoms (Sommer et al. 1986, Fonseca and Bicudo 2008). While succession occurs naturally, other types of disturbance such as pollution may alter the predicted patterns by promoting or inhibiting growth of certain phytoplankton taxa (Pavlic et al. 2005, Carey et al. 2012).

Freshwater ecosystems worldwide receive pollution from personal care products including soaps and detergents that contain surfactants, which act as chemical dispersants and foaming agents (Bu et al. 2013). Surfactants enter aquatic systems via wastewater effluent and surface runoff (Atkinson et al. 2009, Potter et al. 2014). Measured concentrations of nonionic surfactants in surface waters range from 0.013-0.017 mg L⁻¹ (Ghose et al. 2009, Traversa-Soto et al. 2015), though concentrations may be much higher near point sources. One nonionic surfactant group, alkyl polyglucosides (APG), is among the most heavily produced surfactants worldwide; production volume was between 10-50 million lbs in 2015 in the U.S. alone (US EPA, 2016). APG is derived from glucose and fatty alcohols and biodegrades rapidly (within 21 days), which reduces

persistence in the environment and exposure time to organisms (Garcia et al. 1997, Qin et al. 2006).

Despite low toxicity and persistence APG affects freshwater phytoplankton in short-term (acute) and long-term (chronic) toxicity tests, particularly for unicellular green algae (e.g. Steber et al. 1995, Madsen et al. 1996, Duff et al. 2017). Growth reduction of 50% was observed after 72 hours for *Raphidocelis subcapitata* and *Scenedesmus subspicatus* exposed to concentrations of 11 and 6 mg L⁻¹ APG respectively in laboratory tests, suggesting the possibility of differential sensitivity among phytoplankton taxa (Madsen et al. 1996, Steber et al. 1995). Effects of chronic exposure to APG occurred at lower concentrations. Exposing the unicellular green algae *Chlorella* sp. to 2 mg L⁻¹ for seven days caused a 25% decline in cell densities in the lab (Duff et al. 2017). In the field, total phytoplankton abundance decreased by 80% following exposure to 2.5 mg L⁻¹ of APG for one month, however no changes occurred at concentrations <2.5 mg L⁻¹, indicating the potential for a 2 mg L⁻¹ threshold (Riera and Cohen 2016, Duff et al. 2017). Results from these studies suggest that not all Chlorophyte taxa respond the same way to APG exposure.

It is currently unknown how diatoms and cyanobacteria respond to APG, but there is evidence of differential sensitivity to a synthetic surfactant and metals. The nonionic surfactant, C₁₄₋₁₅ alcohol ethoxylate inhibited green algal (*Raphidocelis subcapitata*) growth at concentrations of 50 mg L⁻¹. However, diatom (*Navicula seminulum*) growth was inhibited at concentrations of 10 mg L⁻¹. There were no effects on the cyanobacterium *Microcystis* sp. at concentrations <100 mg L⁻¹ (Payne and Hall 1977). Broise and Palenik (2007) observed taxon-specific responses to copper exposure,

reducing *Synechococcus* sp. populations while simultaneously increasing picoeukaryote populations in microcosms. Species within the same genus (e.g. *Oscillatoria agardhii* vs. *O. redekei*) experienced different rates of growth reduction following exposure to copper sulfate under laboratory conditions (Lüderitz 1988, Lüderitz and Nicklisch 1989). Such taxon-specific responses to chemical exposure can have important repercussions for aquatic communities; if cyanobacteria are less sensitive to APG, they could outcompete other species, ultimately degrading water quality and reducing food availability to zooplankton grazers (Porter and Orcutt 1980, Wilson et al. 2006). Therefore, it is important to assess how different phytoplankton taxa respond to APG exposure in the presence of other species.

Phytoplankton taxa compete for nutrients and light (Yoshiyama et al. 2009), but these interactions (i.e. which species becomes most abundant) can be altered by environmental conditions. The outcome of competition among five marine phytoplankton species (*Phaeodactylum tricornutum* (diatom), *Thalassiosira pseudonana* (diatom), *Skeletonema costatum* (diatom), *Monochrysis lutheri* (phytoflagellate), and *Dunaliella tertiolecta* (single-celled green alga)) was dependent on temperature; *S. costatum* was dominant at 10°C while *D. tertiolecta* was most abundant at 30°C (Goldman & Ryther 1976). High concentrations of nitrogen and phosphorous increased chlorophyte abundance while low nutrient concentrations favored cyanobacteria in a river in China (Zhu et al. 2010). Finally, contamination with polychlorinated biphenyl (PCB) at concentrations of 0.1 µg L⁻¹ did not affect *Thalassiosira pseudonana* growth in pure culture, but reduced growth in the presence of other species (Fisher et al. 1974). Thus, it

is important to consider how APG contamination may affect competitive interactions among phytoplankton taxa.

The focus of this study was to compare the responses of phytoplankton taxa to APG when exposed individually vs. in the presence of other species. I hypothesized that both chemical concentration and the presence of competitors determines how phytoplankton taxa respond to APG contamination. I expected decreased phytoplankton abundance with concentration of APG above 2 mg L^{-1} . I predicted that green algae (*Scenedesmus* sp.) and diatoms (*Navicula* sp.) would be more sensitive to APG than cyanobacteria. Reductions in abundance were also expected to be greatest in the presence of competitors. Results from this study demonstrate that responses of individual phytoplankton species differ when tested alone and in the presence of competitors. Furthermore, our results suggest temperature and light variation in the field is likely an important factor in phytoplankton responses to APG.

METHODS

Culture and Maintenance of Test Species

Cultures of *Microcystis aeruginosa*, *Scenedesmus* sp., and *Navicula* sp. were purchased from Carolina Biological (Burlington, NC) and checked for contaminating algae and grazers, and then maintained in an environmental chamber at 21°C on a 16:8 light:dark cycle (PAR=90 $\mu\text{mol s}^{-1} \text{m}^{-2}$) prior to use in experiments. Cultures were grown in spring water supplemented with Alga-gro freshwater concentrated growth medium (Carolina Biological, Burlington, NC). These three taxa were selected because they are commonly found in freshwater systems worldwide (Jafari and Gunale, 2006). The cyanobacterium *Microcystis aeruginosa* (spherical shape ~5 μm diameter) is colonial in morphology with cells bound within a mucus matrix (Olenina et al. 2006, Ger et al. 2014, Lyu et al. 2017). Some species of *Microcystis* sp., including *Microcystis aeruginosa* used in these studies produce secondary metabolites that can be toxic to zooplankton grazers (Wilson et al. 2006). The green algae *Scenedesmus* sp. (10-20 μm length) forms colonies of 2 to 4 cells and is considered to be high quality food for large bodied grazers such as copepods because of high fatty acid content, but the size and colonial morphology makes it unpalatable to smaller cladocerans (e.g. *Bosmina*) (Brett and Muller-Navarra 1997, Porter and Orcutt 1980). The diatom *Navicula* sp. represents a palatable and high nutrient content food source for grazers of all sizes because it is relatively small (9-12 μm long) and solitary (Lange-Bertalot 2001, Brown 2002).

Phytoplankton Communities

Experimental algal communities were established by mixing volumes with known densities from monocultures of each species. Densities were determined using a flow cytometer (BD Accuri C6, Becton-Dickinson, CA, USA). The flow cytometer allows individual algal cells to pass by lasers that make the cell fluoresce so that cell types can be categorized based on both size and pigment content. Individual cells are counted and plotted, with each species clustering in a separate location from the other species (Figure 1.1). Initial cell densities were chosen to be within ranges of densities measured for each species in freshwater lakes in North America (Makarewicz et al. 1989).

Establishment of APG Treatments

For all experiments, phytoplankton were exposed to 0 (control), 0.01, 0.5, 2 or 10 mg L⁻¹ APG (n=6 for the lab experiments, n=8 for the field experiment). Treatment solutions were mixed using Planteren 2000 (CAS: 68515-73-1, 110615-47-9), an APG compound used in a variety of personal care products and agricultural products (Bu et al. 2013), and spring water (mean pH 8.1). Nutrient concentrations common in spring water range from 0.005-0.05 mg L⁻¹ for phosphate and 5 to 10 mg L⁻¹ for nitrate (USGS, 1996). Concentrations of APG were selected to include each end of the range of concentrations found in surface waters (0.01 and 0.5 mg L⁻¹; Ghose et al. 2009) and a range previously found to decrease freshwater phytoplankton total abundance (2-10mg L⁻¹; Riera and Cohen 2016, Duff et al. 2017). Nominal APG concentrations of 2 and 10 mg L⁻¹ were confirmed using spectrophotometry according to the Anthrone Method, which has a limit of detection of 1 mg L⁻¹ (Buschmann and Wodarczak 1995, Schroder and Ventura 2000).

The average measured concentrations for the 2 and 10 mg L⁻¹ treatments were 1.93 mg L⁻¹ \pm 0.20 SEM and 10.3 \pm 0.28 SEM respectively.

Experimental Design

To determine whether the responses of phytoplankton to APG differ depending on whether species are exposed individually compared to in a community, I conducted a series of laboratory and field experiments.

Community Experiments

In the community experiments known and equal starting densities of the three species of cultured algal taxa were exposed to APG. Experiments were conducted in the lab and field to determine whether natural variation in light and temperature affected phytoplankton community responses to APG. In the lab experiment (n=6), phytoplankton were exposed to APG treatments for one week (5-12 July 2018) in a lab at Georgia Southern University, Statesboro, GA, USA. The community was composed of cyanobacteria (*Microcystis aeruginosa*), green algae (*Scenedesmus* sp.), and diatoms (*Navicula* sp.) with initial average cell density of 3,500 cells ml⁻¹ (*Microcystis* sp.), 4,400 cells ml⁻¹ (*Navicula* sp.), and 4,900 cells ml⁻¹ (*Scenedesmus* sp.). Laboratory microcosms were 250 ml glass beakers containing 200 ml of treatment solution. Beakers were randomized by location and covered with petri dishes to limit evaporation and placed under the light bank (16:8 light:dark cycle, average light intensity of 96.7 \pm 11.5 μ mol m⁻²s⁻¹ and 20° C).

In the community experiment in the field (n=8), microcosms were translucent 20 L floating plastic containers (Cubitainers) (Glibert et al. 2014). Cubitainers were placed

in a pond in Bulloch County, Georgia, USA (32°37'30" N, 81°52'30" W). The pond was approximately 9,000 m² in area, with approximately 3,700 m² at depths greater than 1.3 m (Riera and Cohen 2016). Containers were filled with spring water, APG treatment solution, and *Microcystis* sp., *Navicula* sp., and *Scenedesmus* sp. in equal proportions. Initially, average cell density of each species was 7.4×10^3 cells ml⁻¹. Mean temperature on day 7 was 30.1±0.07 °C, and mean pH was 8.2±0.03. Cubitainers were neutrally buoyant, remaining submerged just under the surface of the pond, and anchored with stakes into the bottom of the pond in two parallel rows of 20 with 0.5 m between them. Cubitainers were approximately 20 m from the edge to avoid shading by trees.

Individual species laboratory experiments

Individual species experiments (n=6) were conducted under the same conditions as the laboratory community experiment between 13 October and 17 November 2018. The species, cyanobacteria (*Microcystis aeruginosa*), green algae (*Scenedesmus* sp.), and diatom (*Navicula* sp.) initial average cell densities for each experiment were 3.3×10^4 cells ml⁻¹. Laboratory microcosms were 250 ml glass beakers containing 200 ml of treatment solution. Beakers were randomized by location and covered with petri dishes to limit evaporation and placed under the light bank (16:8 light:dark cycle, average light intensity of $96.7 \pm 11.5 \mu\text{mol m}^{-2}\text{s}^{-1}$ and 20° C).

Sampling Experiments for Phytoplankton Abundance

Flow Cytometry

Abundance of each species was measured as cell density using flow cytometry. For laboratory experiments, microcosms were first homogenized and a 1 ml subsample taken. Each subsample was vortexed to re-suspend cells before analysis (BD Accuri C6, Becton-Dickinson, CA, USA). For the field experiment, cubitainers were homogenized before 200 ml water samples were collected and transported in a dark cooler to the laboratory for subsequent processing.

*Chlorophyll *a* Concentration*

Chlorophyll *a* concentration was determined as an additional measure of abundance. Homogenous water samples (100 ml for all experiments) were filtered through glass microfiber filters (Whatman GF/F, nominal pore size 0.7 μ m) to collect phytoplankton cells. Pigments from the collected cells were extracted in 90% acetone in the dark at -20°C for 24 hours followed by analysis using a Trilogy fluorometer (Turner Designs, CA, USA) according to EPA protocol 445.0 (Arar and Collins 1997).

Statistical Analysis

Differences in cell density and chlorophyll *a* concentration across APG treatments were analyzed using one-way ANOVA. Pairwise comparisons between treatments were done using Tukey HSD post-hoc tests. Prior to analysis, all data were transformed using log response ratios (LRR) before being tested for normality using the Shapiro-Wilk *W* test, and homogeneity of variances using Levene's test. LRR was utilized as a way to

compare direction responses of phytoplankton to APG concentrations between experiments conducted at different times with different initial cell densities. Individual species experiments started with the same cell densities, so comparisons could be done without transformation. This transformation has been used in previous studies to compare phytoplankton results from experiments conducted at different times (Duff et al. 2017).

LRR is useful for phytoplankton experiments considering that cultures of the same species of phytoplankton grown in the same conditions can vary in quality. Data were also analyzed as percent change from initial in order to compare when the initial cell densities were not equal. For this transformation, initial cell densities were subtracted from final cell densities and then divided by final cell densities and multiplied by 100 to turn the value into a percentage. Data were then tested for normality and homogeneity of variances before analysis. One-way ANOVA was used to determine significant differences between treatment groups. Post-hoc tests were done using Tukey HSD post-hoc test. All tests were performed using JMP, Pro 13 (SAS Institute Inc., Cary, NC). Changes in species composition in response to APG exposure for the communities in both the lab and field microcosm experiments were determined from Bray-Curtis resemblance matrices followed by analysis using one-way permutational multivariate analysis of variance, with APG treatment as the factor (PERMANOVA+ add on; PRIMER-E v.7, Plymouth Marine Laboratory, U.K.) (Clarke and Gorley 2006).

RESULTS

Community Experiments

Lab Community

Exposure to APG altered phytoplankton community composition under laboratory conditions (PERMANOVA, $F_{4,29} = 8.21$, $p = 0.0001$) (Table 1.1). Despite all three species starting in equal proportions *Scenedesmus* sp. comprised 3% of the community, while *M.aeruginosa* and *Navicula* sp. were present in approximately equal proportions in the 0, 0.01, and 0.5 mg L⁻¹ treatments (Figure 1.2A). In contrast, the 2 mg L⁻¹ treatment was dominated by *Navicula* sp. due to a 50% reduction in *M.aeruginosa* cell density. Only the 10 mg L⁻¹ treatment was composed of equal proportions of each species, although the total cell density decreased by about 90% compared to control (Figure 1.2A). LRR results suggest that *M.aeruginosa* cell density was reduced most in the 2 and 10 mg L⁻¹ treatments (Figure 1.3A). *Navicula* sp. cell densities also decreased in the 2 and 10 mg L⁻¹ treatments, with the largest log decrease in the 10 mg L⁻¹ treatment. In contrast, *Scenedesmus* sp. had little to no response in the 2 mg L⁻¹ treatment showed a log increase in cell density compared to controls. Percent change from initial cell densities for *M.aeruginosa* (ANOVA, $F_{4,29} = 17.4$, $p = <0.0001$) decreased with increasing concentration, while *Scenedesmus* sp. experienced an increase in percent change from initial in the 10 mg L⁻¹ treatment (ANOVA, $F_{4,29} = 22.6$, $p = <0.0001$) (Figure 1.4A). Total cell density also decreased by roughly 80% from 15.7×10^4 cells ml⁻¹ in controls to 2.7×10^4 cells ml⁻¹ in the 10 mg L⁻¹ treatment (Figure 1.2B). Total phytoplankton abundance (as chlorophyll-*a* concentration) generally agreed with cell density measurements. Chlorophyll-*a* concentration in APG treatments of 0.5 mg L⁻¹ and above

decreased compared to controls coinciding with losses of *Microcystis* sp. and *Navicula* sp. (ANOVA, $F_{4,29} = 16.58$, $p = <0.0001$) (Table 1.3A; Figure 1.2C). In the 0.5 mg L⁻¹ treatment chlorophyll-*a* concentration differed from control while cell density did not (Figure 1.2A, Figure 1.2 C). LRR results for chlorophyll-*a* concentration indicated that the greatest negative responses to APG were in the 2 and 10 mg L⁻¹ treatment (Figure 1.3C).

Field Community

Community responses to APG treatments in field microcosms were similar to those observed in the laboratory (PERMANOVA, $F_{4,39} = 3.72$, $p = 0.0026$) (Table 1.1B; Figure 1.2B). While no differences in cell density from controls occurred in the community until 2 mg L⁻¹ under laboratory conditions, exposure to 0.5 mg L⁻¹ triggered differences in community composition (Figure 1.2A,B). Differences in community composition in the 0.5 mg L⁻¹ treatment were due to decreased *M.aeruginosa* cell density. As in the lab experiment, *Scenedesmus* sp. composed the smallest portion of the community (~5-10%), while the majority of the community in the 0 and 0.01 mg L⁻¹ treatments consisted of relatively equal proportions of *M.aeruginosa* and *Navicula* sp. (Figure 1.2B). For all species, LRR responses did not differ between treatments (Table 1.3B) LRR responses for this experiment for *M.aeruginosa* and *Navicula* sp. follow a similar pattern of negative responses with increasing APG treatments, while *Scenedesmus* sp. had positive responses to APG in the field (Figure 1.3B). As percent change from initial, APG concentrations of 2 and 10 mg L⁻¹ generally had the lowest percent changes (Figure 1.4B). *M.aeruginosa* (ANOVA, $F_{4,39} = 3.79$, $p = 0.011$) and *Navicula* sp. (ANOVA, $F_{4,39} = 4.42$, $p = 0.005$) had the largest percent increases from initial. While

APG-induced reductions in cell density in field microcosms were mostly due to loss of *Navicula* sp. (~50%) (Figure 1.2B), decreases in the lab were mostly due to *M.aeruginosa* (~50%) (Figure 1.2A). In both the lab and field experiments, chlorophyll-*a* concentration decreased most in the 2 and 10 mg L⁻¹ treatments (ANOVA, $F_{4,39} = 9.50$, $p = <0.0001$) (Table 1.3B; Figure 1.2C,D). LRR responses confirm that chlorophyll-*a* was influenced by APG (ANOVA, $F_{4,39} = 9.50$, $p = <0.0001$) (Table 1.4). The 2 and 10 mg L⁻¹ treatments resulted in overall decreases in chlorophyll-*a* LRR values, and the effects of these treatments were about double those of the 0.01 and 0.5 mg L⁻¹ treatment (Figure 1.3D). As percent change, the 2 and the 10 mg L⁻¹ concentrations had greater effects on chlorophyll-*a* than other treatments (ANOVA, $F_{4,39} = 10.03$, $p = <0.0001$).

Individual Species Experiments

Microcystis aeruginosa

Nonlinear decreases in *M.aeruginosa* cell density occurred with increasing APG concentration when alone (ANOVA, $F_{4,29} = 10.9$, $p = <0.0001$) (Table 1.2A,B). When exposed individually only APG concentrations ≥ 0.5 mg L⁻¹ had an effect, and the effect was not enhanced by increasing APG concentration (Figure 1.5A). Effects of APG on *M.aeruginosa* differed in the presence of competitors (ANOVA, $F_{4,29} = 17.4$, $p = <0.0001$). With competitors, *M.aeruginosa* was more sensitive to APG with decreased cell density occurring at 0.01 mg L⁻¹ with increasing effect in the 2 and 10 mg L⁻¹ treatments (Figure 1.2A). LRR results for cell density indicate that while significant negative effects occurred when alone in lab at 0.5 mg L⁻¹, that the greatest negative responses occurred at 2 and 10 mg L⁻¹ in community tests (Figure 1.6A). As percent change from initial, *M.aeruginosa* cell density increased in all treatments, with the

smallest percent increases occurring in concentrations $\geq 0.5 \text{ mg L}^{-1}$ (ANOVA, $F_{4,29} = 10.98$, $p = <0.0001$) (Figure 1.7A). In the individual species test, effects of APG on chlorophyll-*a* concentrations occurred in a linear pattern similar to cell density effects, however the 0.01 mg L^{-1} treatment did affect chlorophyll-*a* concentration (ANOVA, $F_{3,23} = 12.25$, $p = <0.0001$) (Table 1.4C; Figure 1.7B). Concentrations $\geq 0.5 \text{ mg L}^{-1}$ all had similar effects on chlorophyll-*a* concentration and cell density. APG exposure also decreased overall chlorophyll-*a* concentration by ~43-90% in the 0.01, 0.5, 2 and 10 mg L^{-1} treatments (Figure 1.7B). LRR for chlorophyll-*a* show that all treatment groups caused a negative chlorophyll-*a* response, where the greatest negative response occurred in the 10 mg L^{-1} treatment (Figure 1.7B). As percent change, chlorophyll-*a* was also affected with the largest effect occurring in concentrations greater than 0.5 mg L^{-1} (ANOVA, $F_{4,29} = 12.25$, $p = <0.0001$). The 0.01 mg L^{-1} treatment also significantly reduced chlorophyll-*a* compared to controls.

Navicula sp.

There was a nonsignificant pattern of decreased cell density in the presence of APG and competitors (ANOVA, $F_{4,29} = 2.0$, $p = 0.12$) (Table 1.2A), especially in the 10 mg L^{-1} treatment where cell density was reduced by 90% compared to controls (Figure 1.2A). A pattern for reductions of cell density at concentrations 2 mg L^{-1} or greater was also observed, but low abundances and high variability likely led to non-significant results. However, when exposed to APG alone, *Navicula* density was reduced by 40-50% in the 0.5, 2, and 10 mg L^{-1} treatments compared to controls, with an 80% reduction at 2 mg L^{-1} (ANOVA, $F = 32.4$, $p = <0.0001$) (Table 1.2B; Figure 1.8A). LRR results show that when alone, *Navicula* sp. had its most negative response to 2 mg L^{-1} APG (Figure

1.9A). When tested in the presence of competitors, the 0.01 mg L⁻¹ treatment caused more negative responses to APG than the 0.5 or 2 mg L⁻¹ treatments (Figure 1.8A). As percent change from initial, the largest reductions in percent change occurred in the 2 and 10 mg L⁻¹ treatments (ANOVA, $F_{4,29} = <0.0001$) (Figure 1.10A). Reductions in cell density corresponded with chlorophyll-*a* concentration, where the most negative responses occurred at 2 and 10 mg L⁻¹ (Figure 1.10B). Reductions in cell density occurred at 0.5 mg L⁻¹, but there were no effects on chlorophyll-*a* concentration until 2 mg L⁻¹. The 2 and 10 mg L⁻¹ treatments decreased chlorophyll-*a* concentration by 86 and 96% respectively (Figure 1.8B). Chlorophyll-*a* LRR showed a threshold effect, where the 2 and 10 mg L⁻¹ concentrations had the most negative response (Figure 1.9 B). Results for percent change indicated that the 2 and 10 mg L⁻¹ treatments had greatest reductions of chlorophyll-*a* (ANOVA, $F_{3,29} = 12.1$, $p = <0.0001$) (Figure 1.10B).

Scenedesmus sp.

Scenedesmus sp. cell density increased compared to controls in response to APG alone and in the presence of competitors under laboratory conditions (ANOVA, $F_{4,39} = 106.73$, $p = <0.0001$) (ANOVA, $F_{4,29} = 22.65$, $p = <0.0001$) (Table 1.2). However, overall *Scenedesmus* sp. cell densities were low compared to the other two species in all three experiments. In particular, 10 mg L⁻¹ reduced the cell densities of both other species (Figure 1.2A). *Scenedesmus* sp. was the least dominant of the species and had lower cell densities than *Navicula* sp. or *M.aeruginosa*. LRR results for the experiments indicate that *Scenedesmus* sp. cell densities were less affected by APG when alone (Figure 1.12A). When in the presence of competitors, *Scenedesmus* sp. had negative LRR cell

density responses to 0.01 and 0.5 mg L⁻¹. As percent change from initial, *Scenedesmus* sp. has the largest percent increase in cell density in the 10 mg L⁻¹ treatment (ANOVA, $F_{4,29} = <0.0001$) (1.13A). While cell density LRR had positive responses, chlorophyll-*a* concentration exhibited the opposite pattern of negative responses for all treatments, being an order of magnitude lower at 10 mg L⁻¹ (ANOVA, $F_{3,23} = 132.91$, $p = <0.0001$) (Table 1.5C; Figure 1.13B). As LRR, chlorophyll-*a* was had a negative response for all treatments and there were no differences from one another (Figure 1.12). Percent change results for chlorophyll-*a* indicate that only the 10 mg L⁻¹ treatment reduced chlorophyll-*a* concentration (ANOVA, $F_{4,29} = 22.5$, $p = <0.0001$).

DISCUSSION

Phytoplankton species responses to APG were hypothesized to be determined by both the concentration of APG, and the presence of competitors. Decreased phytoplankton abundance with exposure to APG concentrations $\geq 2 \text{ mg L}^{-1}$ were expected based on the literature (e.g. Riera and Cohen 2016, Duff et al. 2017) and were largely reflected in the results from the present study. In some instances, there were effects at concentrations below 2 mg L^{-1} . For example, in the field community experiment, there was a significant change in community composition at 0.5 mg L^{-1} . This was likely a result of natural variation in light and temperature with abiotic factors varying more in the field than in the lab, where phytoplankton responses to APG could also be driven by their temperature optimums (Sommer et al. 1986). *M.aeruginosa* tends to do better at higher temperatures than the other two phytoplankton species used in this study. There were also significant effects on chlorophyll-*a* at 0.01 and 0.5 mg L^{-1} in the *M.aeruginosa* experiment. It is possible that the temperature conditions in the laboratory were not favorable for *Microcystis* sp. since it typically grows better in higher temperatures (Ryther and Sanders 1980).

Results from the current studies support the idea of differential sensitivity to APG amongst species. Phytoplankton taxa considered to be high quality food for grazers (*Navicula* sp.) are sensitive to APG, specifically in the field with natural temperature variation. Reductions of *Navicula* sp. cell densities in the field is not surprising, considering diatom cell densities are typically low during warm summer months (Fonseca and Bicudo 2008). It was predicted that *Scenedesmus* sp. and *Navicula* sp. would be more sensitive to APG. While *Scenedesmus* sp. generally did poor in all

treatments, it is difficult to say whether it matched the prediction of being more sensitive to APG was supported. However, there is literature suggesting *Scenedesmus* sp. is a poor competitor in general. A competition study comparing *Chlorella* sp., *Scenedesmus* sp., and *Microcystis* indicated that *Scenedesmus* sp. was the worst competitor for light (Huisman et al. 1999). In this study, when *Chlorella* sp. and *Microcystis* sp. were introduced to monocultures of *Scenedesmus* sp., both were able to overtake the culture, becoming the dominant species. But, when *Scenedesmus* sp. was added to monocultures of *Chlorella* sp. and *Microcystis* sp., it was not able to take over the cultures.

Scenedesmus sp. being a poor competitor for light could explain why even when each species began in the same proportions, that *Scenedesmus* sp. fell out of dominance in all treatments including controls. It was predicted that *Scenedesmus* sp. and *Navicula* sp. would be more sensitive to APG than *Microcystis* sp. based on previous studies of synthetic surfactants (Payne and Hall 1977). In individual species tests, *Microcystis* sp. and *Navicula* sp. cell densities decreased after seven days of exposure to APG, with the 2 and 10 mg L⁻¹ treatments having the greatest effect. In contrast, *Scenedesmus* sp. cell density increased in 10 mg L⁻¹ APG after seven days of exposure, though chlorophyll-*a* values for this treatment were an order of magnitude lower than controls and overall *Scenedesmus* sp. cell densities were low. This increase in *Scenedesmus* cell density resulting from APG could have been due to the degradation of APG into glucose and fatty alcohols, which can then oxidize into fatty acids (Eichhorn & Knepper 1999). Added glucose from APG degradation could be providing phytoplankton cells with a source of carbon that can be used for growth during periods when nutrient levels may be low (Heredia-Arroyo et al. 2010). Similar increases in green algal cell density for

Chlorella sp. have been observed when APG treatments are applied in tandem with nutrient additions (Duff et al. 2017). A doubling of *Chlorella* sp. cell dry weight in response to glucose addition has also been recorded (Cheirsilp and Torpee 2012). Further study of the effects of APG on green algal species is needed to better understand the mechanism behind increases in cell density or chlorophyll-*a*. It is also possible that the response of *Scenedesmus* sp. is because it is not a good competitor for light compared to the other species tested (Huisman et al. 1999).

Changes in community composition were attributed to reductions of *Navicula* sp. and *M.aeruginosa*, the two dominant taxa. In the field community experiment, decreases in cell density were mostly due to decreases in *Navicula* sp., while decreases in the lab community were from *M.aeruginosa*. Phytoplankton taxa compete for nutrients and light these competitive interactions can be intensified when contaminants are present (Goldman and Ryther 1976, Yoshiyama et al. 2009, Zhu et al. 2010). In this case, the response was likely due to the temperature conditions; the lab experiment was maintained at ~20° C, while temperatures in the field experiment ranged from ~25-30° C.

Cyanobacteria typically dominate phytoplankton communities during oligotrophic summer conditions, while diatoms are abundant at cooler temperatures (Sommer et al. 1986, Fonseca and Bicudo 2008). The effects of temperature on changes in on freshwater phytoplankton community composition in the presence of APG is important to understand, particularly as global temperatures continue to increase.

APG also has the potential to affect cell quality. While APG resulted in increased cell density for all experiments, cell quality may have been compromised. Decreases in chlorophyll-*a* content could alter the food quality of the phytoplankton to zooplankton

grazers, potentially affecting zooplankton growth or reproduction (Kimmerer et al. 2005). Chlorophyll-*a* content is an indication of how productive cells are being, and low production would lower the nutritional quality the cells can provide to grazers.

Shifts in phytoplankton community composition from APG that lead to reduced abundance of diatoms could result in a reduced amount of food available to grazers, particularly during summer months when diatom and green alga densities are already low (Fonseca and Bicudo 2008). Communities composed of high densities of cyanobacteria, such as *Microcystis* sp., could potentially result in decreased freshwater zooplankton abundance (Wilson et al. 2006, Zhu et al. 2013, Ger et al. 2014, Lyu et al. 2017). Future studies should focus on comparing responses of green alga to responses of diatoms or cyanobacteria after longer durations to better understand species-specific responses of cell density and chlorophyll responses to APG. With longer exposure times, it is possible that the shift in community could become more extreme, with the potential to affect food quality or availability to higher trophic levels. Shifts in phytoplankton community composition caused by APG have the potential to alter food resource availability to grazers, as colony-forming, cyanobacteria like *Microcystis* sp. are less preferred as food and potentially toxic to grazers in high enough density (Wilson et al. 2006, Fonseca and Bicudo 2008). To see changes in food availability you would likely need longer than one week. This longer time frame would allow for reductions in food quality to begin to influence grazer populations, which could ultimately influence fish. Since no grazers were present in these experiments, it will be important to determine how grazing may influence APG toxicity to phytoplankton.

Table 1.1. ANOVA results for differences in cell density after seven days of APG treatment in community test under laboratory conditions (n=6)(A), community test under field conditions (n=8)(B), and individual species tests (C)(n=6).

A. Community in laboratory

Cell Type	Df (Effect, Total)	F	<i>p</i>
<i>Scenedesmus</i> sp.	4,29	22.6522	<0.0001*
<i>Navicula</i> sp.	4,29	2.0094	0.1239
<i>Microcystis</i> sp.	4,29	17.4293	<0.0001*

B. Community in field

Cell Type	Df (Effect, Total)	F	<i>p</i>
<i>Scenedesmus</i> sp.	4,39	2.4586	0.0635
<i>Navicula</i> sp.	4,39	4.4286	0.0053*
<i>Microcystis</i> sp.	4,39	3.7936	0.0115*

C. Individual Species

Cell Type	Df (Effect, Total)	F	<i>p</i>
<i>Scenedesmus</i> sp.	4,29	106.7317	<0.0001*
<i>Navicula</i> sp.	4,29	32.4729	<0.0001*
<i>Microcystis</i> sp.	4,29	10.9883	<0.0001*

Table 1.2. LRR ANOVA results for differences in cell density after seven days of APG treatment in community test under laboratory conditions (n=6)(A), community test under field conditions (n=8)(B), and individual species tests (C)(n=6).

A. Community in laboratory

Cell Type	Df (Effect, Total)	F	<i>p</i>
<i>Scenedesmus</i> sp.	3,23	40.02	<0.0001*
<i>Navicula</i> sp.	3,23	2.95	0.0572
<i>Microcystis</i> sp.	3,23	15.59	<0.0001*

B. Community in field

Cell Type	Df (Effect, Total)	F	<i>p</i>
<i>Scenedesmus</i> sp.	3,31	1.12	0.3541
<i>Navicula</i> sp.	3,31	0.692	0.5641
<i>Microcystis</i> sp.	3,31	1.70	0.1890

C. Individual Species

Cell Type	Df (Effect, Total)	F	<i>p</i>
<i>Scenedesmus</i> sp.	3,23	53.28	<0.0001*
<i>Navicula</i> sp.	3,23	63.23	<0.0001*
<i>Microcystis</i> sp.	3,23	5.87	0.0048*

Table 1.3. ANOVA results for comparisons of chlorophyll-*a* concentration responses to APG treatment after seven days of exposure during a community test under laboratory conditions (n=6)(A), under field conditions (n=8)(B), and for individual species tests (n=6)(C).

A. Community in laboratory

Df (Effect,Total)	F	<i>p</i>
4,29	16.5852	<0.0001*

B. Community in field

Df (Effect,Total)	F	<i>p</i>
4,39	9.5029	<0.0001*

C. Individual Species

Cell type	df (Effect,Total)	F	<i>p</i>
<i>Scenedesmus</i> sp.	4,29	22.5097	<0.0001*
<i>Navicula</i> sp.	4,29	25.8959	<0.0001*
<i>Microcystis</i> sp.	4,29	12.2533	<0.0001*

Table 1.4. LRR ANOVA results for comparisons of chlorophyll-*a* concentration responses to APG treatment after seven days of exposure during a community test under laboratory conditions (n=6)(A), under field conditions (n=8)(B), and for individual species tests (n=6)(C).

A. Community in laboratory

Df (Effect,Total)	F	<i>p</i>
3,23	3.17	<0.0001*

B. Community in field

Df (Effect,Total)	F	<i>p</i>
3,31	10.51	<0.0001*

C. Individual Species

Cell type	df (Effect,Total)	F	<i>p</i>
<i>Scenedesmus</i> sp.	3,23	132.91	<0.0001*
<i>Navicula</i> sp.	3,23	69.30	<0.0001*
<i>Microcystis</i> sp.	3,23	18.94	<0.0001*

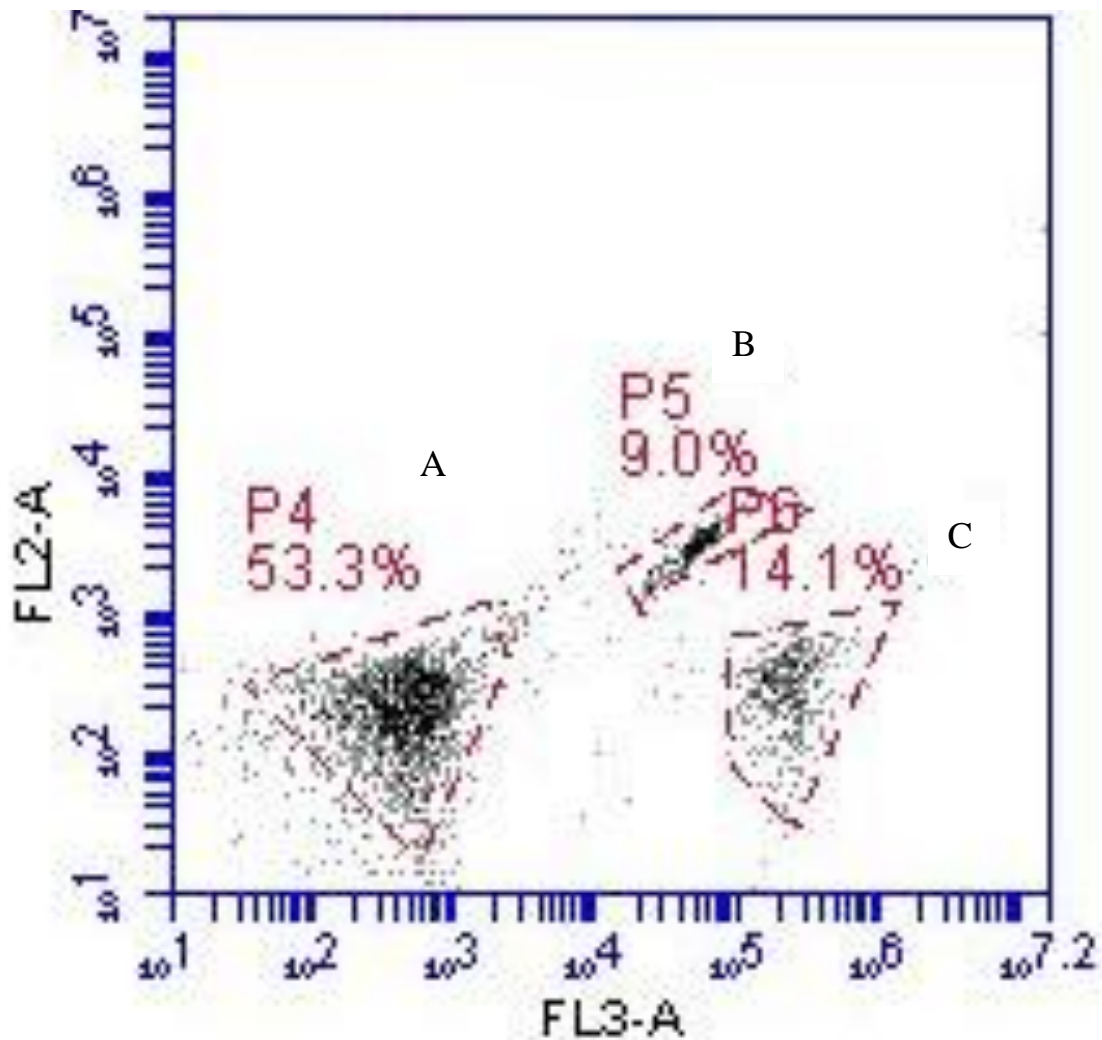


Figure 1.1. This is an example of a plot from the flow cytometer showing the profiles of the three species where each individual point is a cell. The group labeled **A** represents the profile of *Scenedesmus* sp., **B** *Microcystis aeruginosa*, and **C** *Navicula* sp.

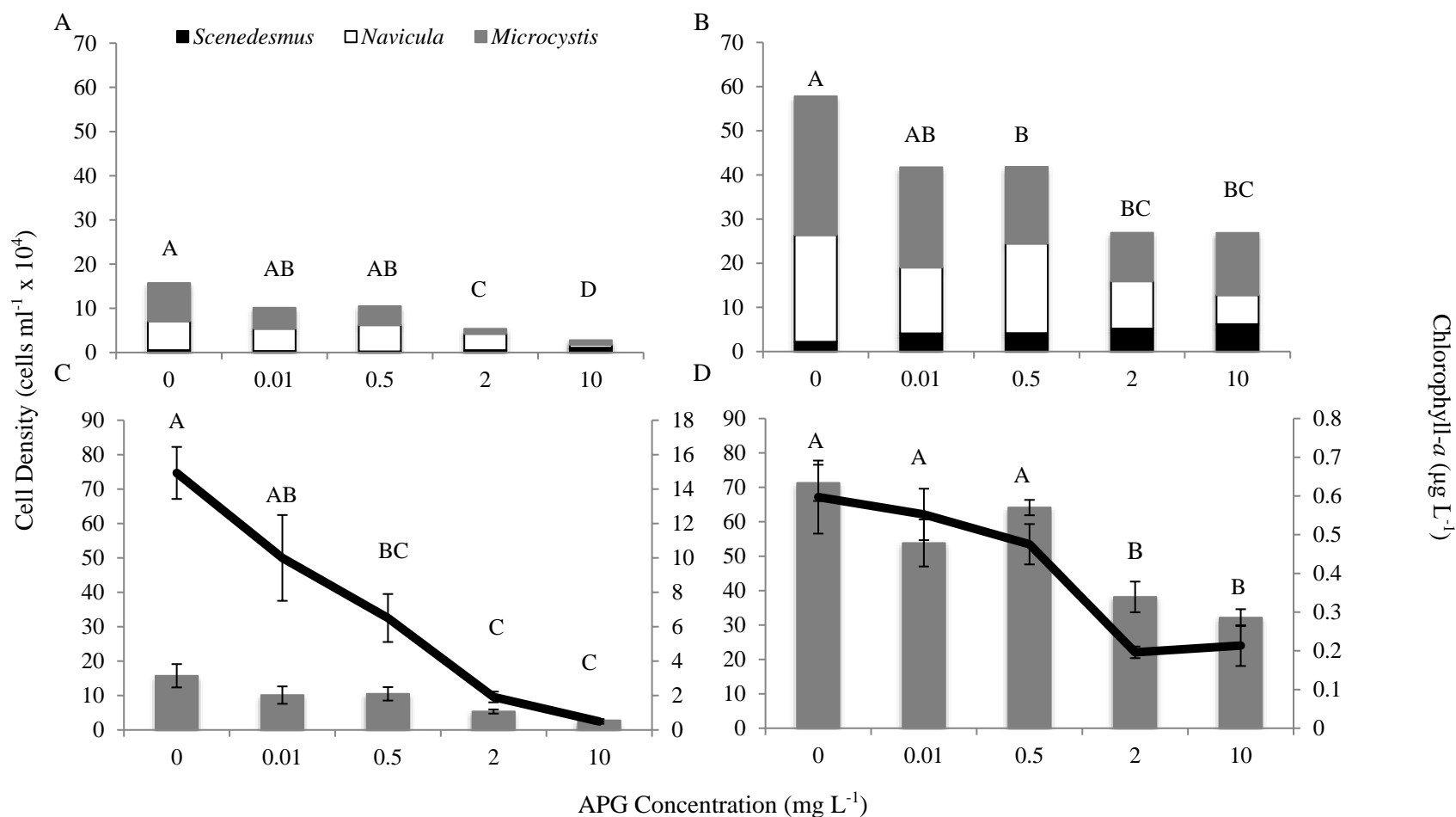


Figure 1.2. Mean cell densities of *Scenedesmus* sp., *Navicula* sp., and *Microcystis aeruginosa* after seven days of exposure to 0, 0.01, 0.5, 2 or 10 mg L⁻¹ in laboratory (n=6)(A) and field (n=8)(B) test, and mean chlorophyll-a concentrations (lines) and total cell densities (bars) for laboratory (C) and field (D) tests. Error bars are \pm one standard error of the mean (SEM). Different letters represent Tukey HSD differences from controls.

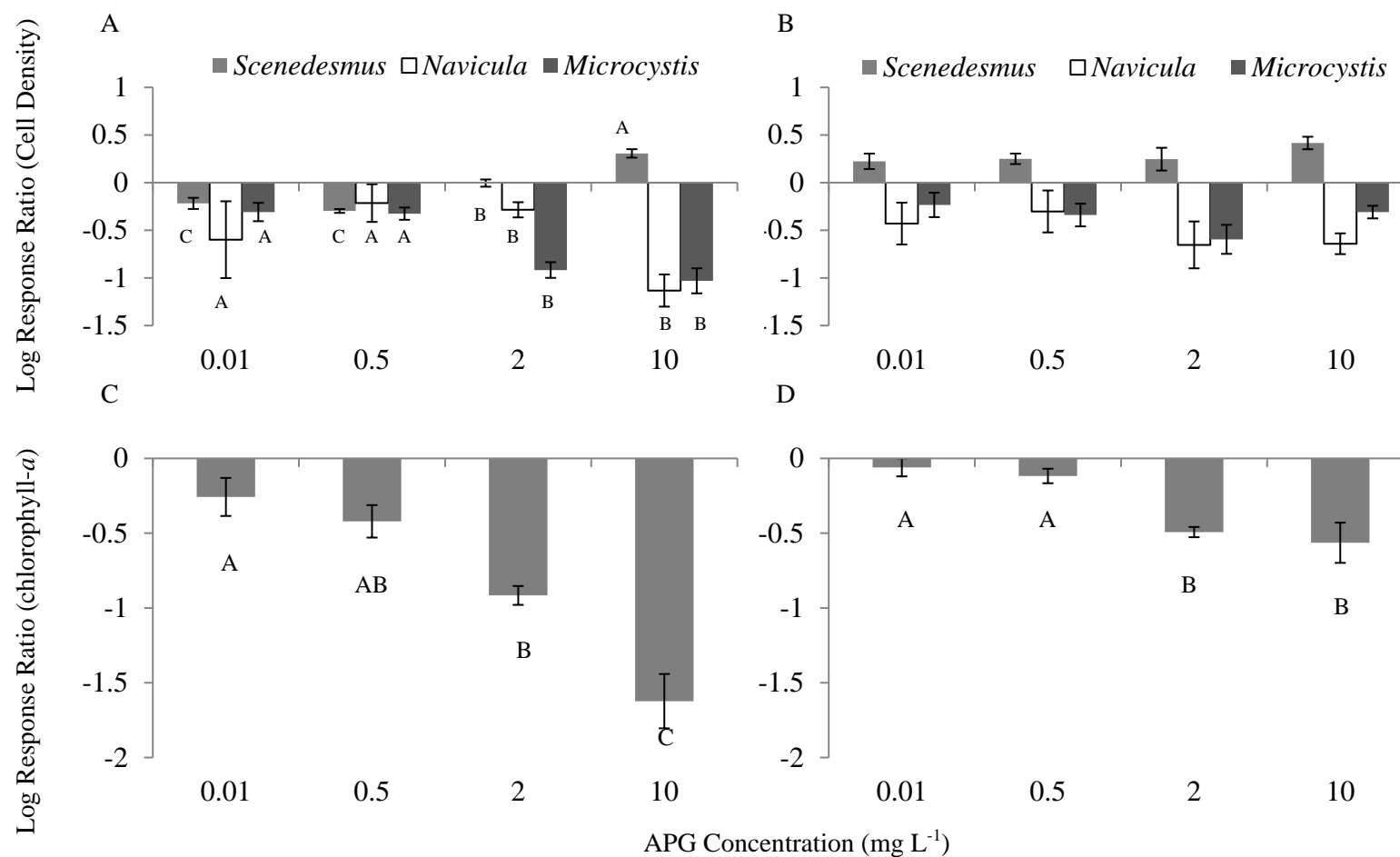


Figure 1.3. Mean Log Response Ratios for cell density for the community in lab (n=6)(A) and field experiments (B) and mean log response ratios for chlorophyll-*a* concentration in the lab (n=6)(C) and field (n=8)(D). Pairwise comparisons of the Tukey HSD post-hoc tests are indicated by letters where treatments not connected by the same letters are different from each other.

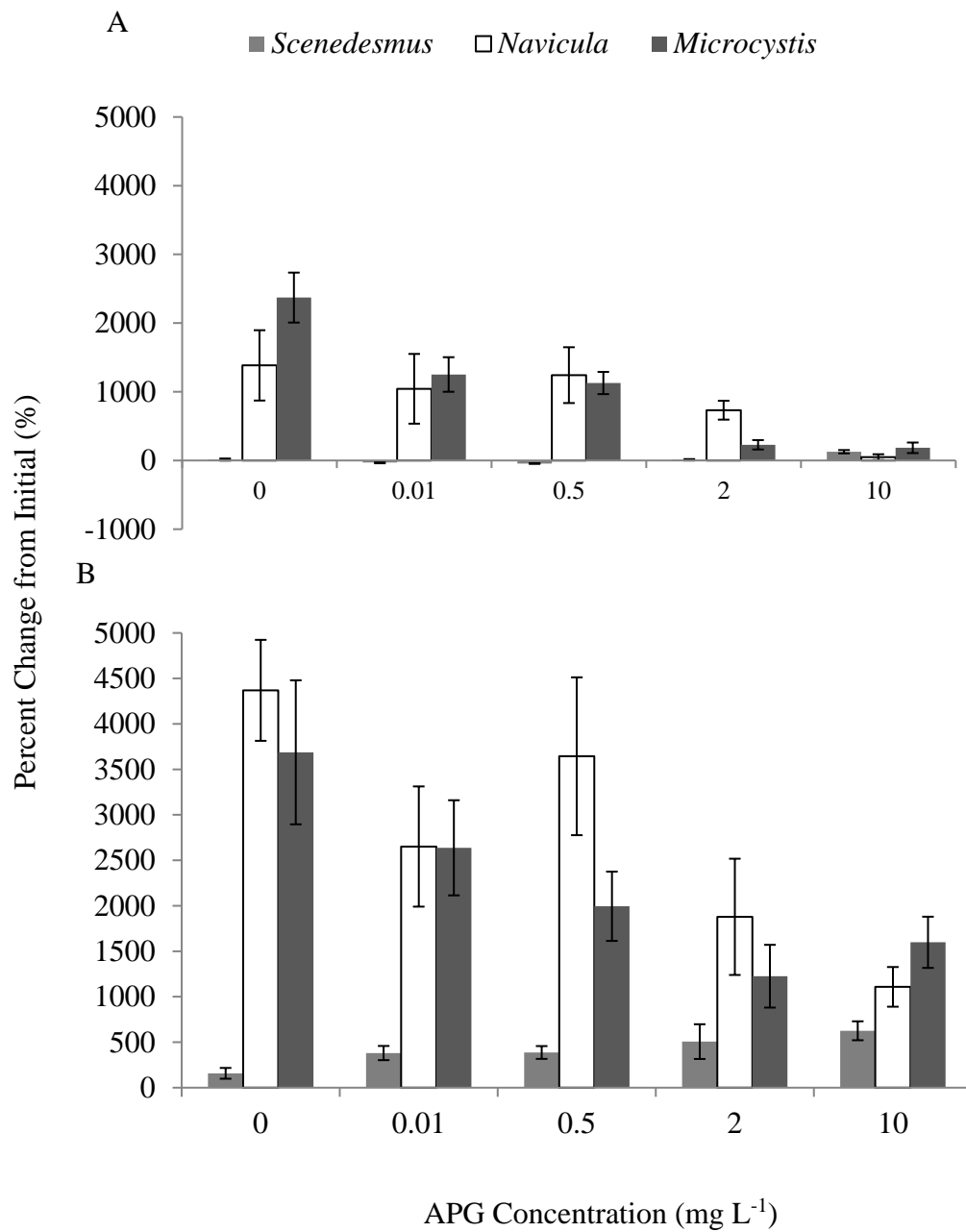


Figure 1.4. Mean percent change from initial cell density readings to final for each of the three species in community tests in the lab (n=6)(A) and field (n=8)(B). Error bars are \pm one SEM.

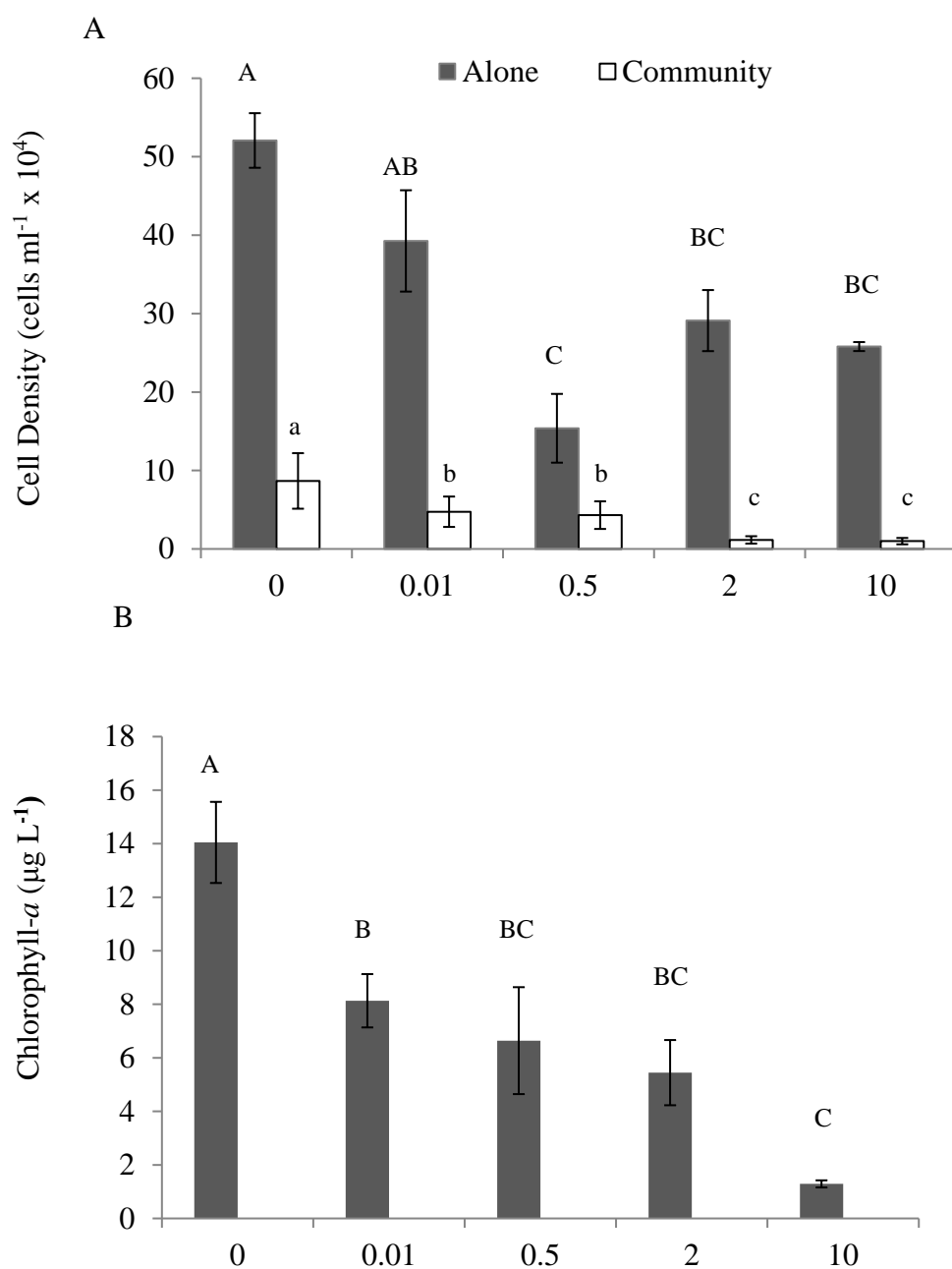


Figure 1.5. Mean cell densities of *Microcystis aeruginosa* (A) and chlorophyll-a concentration (B) after seven days of exposure to 0, 0.01, 0.5, 2 and 10 mg L⁻¹ APG in individual species or community tests (n=6). Error bars are \pm one SEM. Tukey HSD comparisons for individual species are indicated by upper-case letters, while comparisons for the community are indicated by lowercase letters. Treatments not connected by the same letter are significantly different.

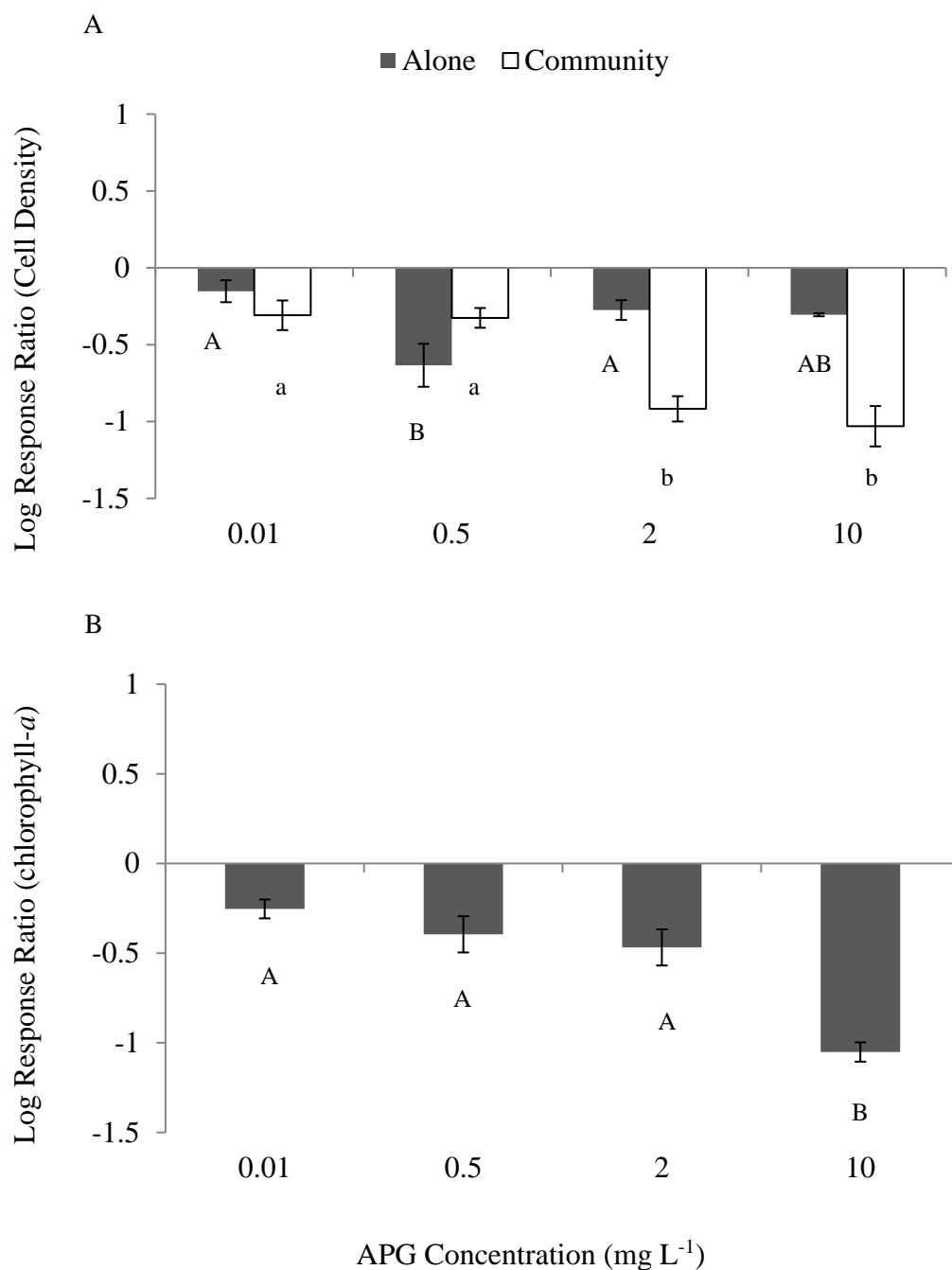


Figure 1.6. Mean LRR for *Microcystis aeruginosa* cell density in laboratory tests (n=6) alone and in community (A) and mean chlorophyll-a LRR (B). Letters represent Tukey HSD post-hoc comparisons. Treatment groups not connected by the same letter are significantly different from one another. Uppercase letters show differences in individual species experiments, while lowercase letters show differences for community experiments. Error bars are \pm one SEM.

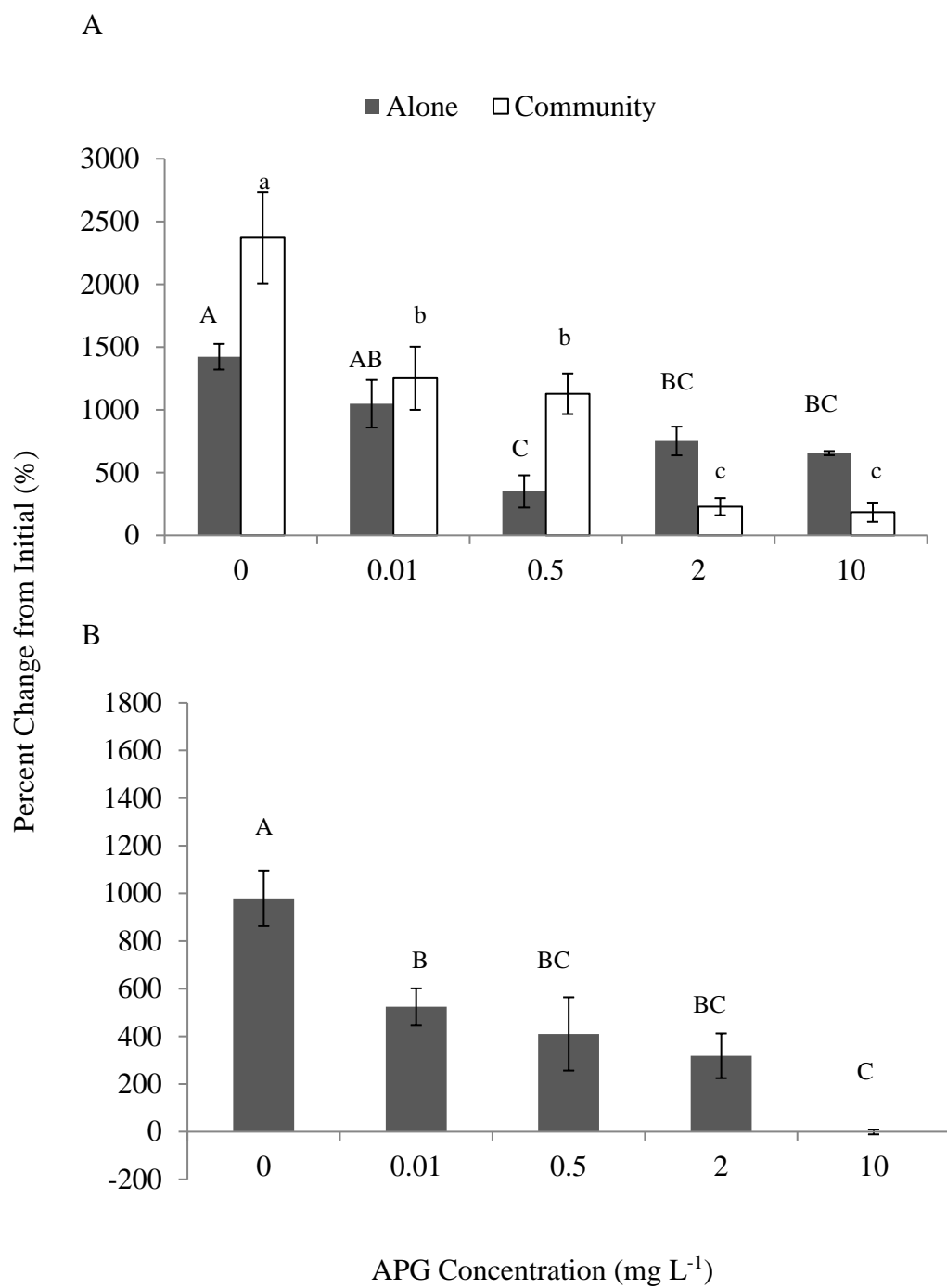


Figure 1.7. Mean percent change from initial cell density readings for *Microcystis aeruginosa* for cell density alone (n=6) and when in community (n=6)(A) and chlorophyll-a concentration for individual species test (B). Error bars are \pm one SEM.

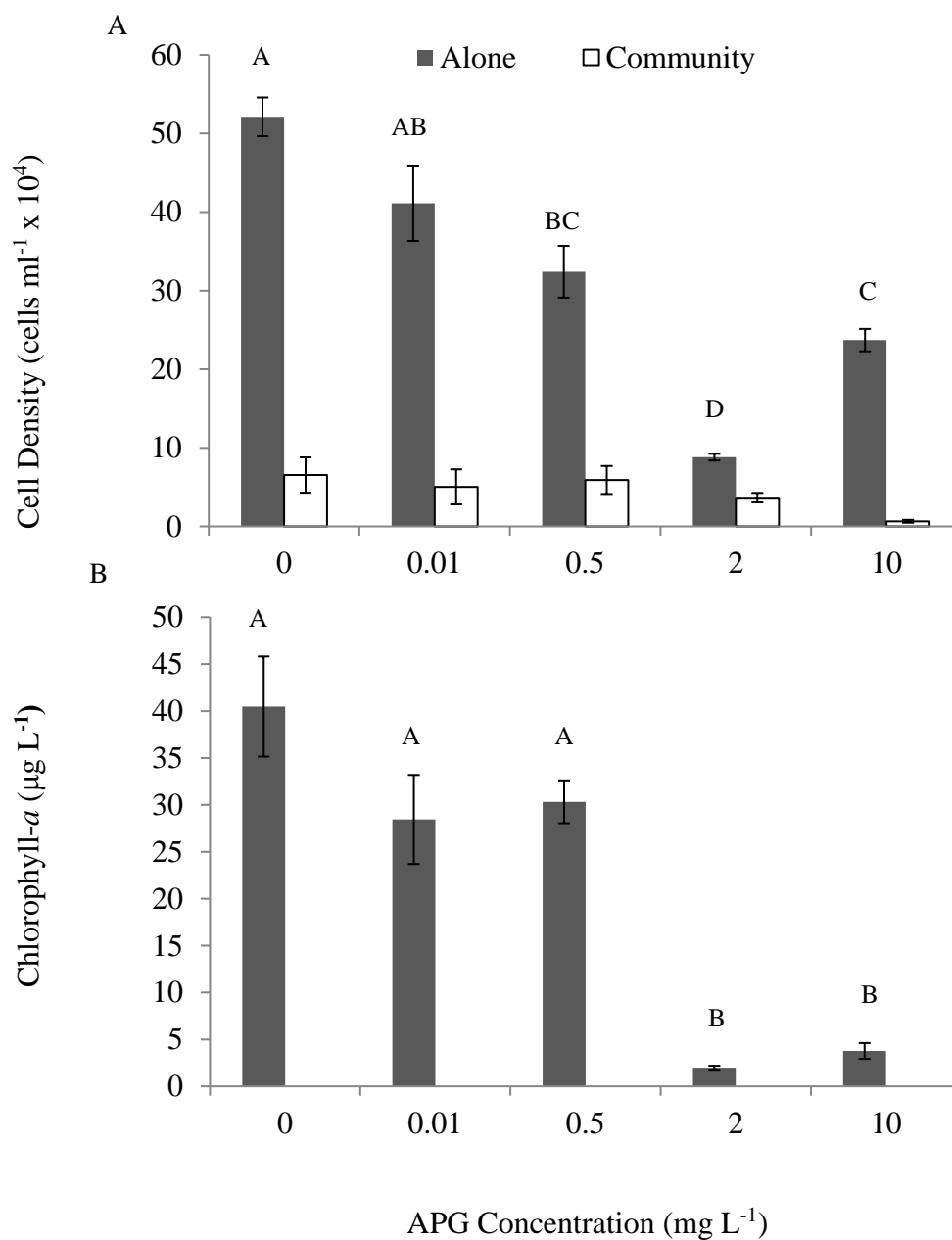


Figure 1.8. Mean cell densities of *Navicula* sp. (A) and chlorophyll-*a* concentration (B) after seven days of exposure to 0, 0.01, 0.5, 2 and 10 mg L⁻¹ APG in individual species or community tests (n=6). Error bars are \pm one SEM. Tukey HSD post-hoc comparisons for individual species are indicated by letters. Treatments not connected by the same letter are significantly different.

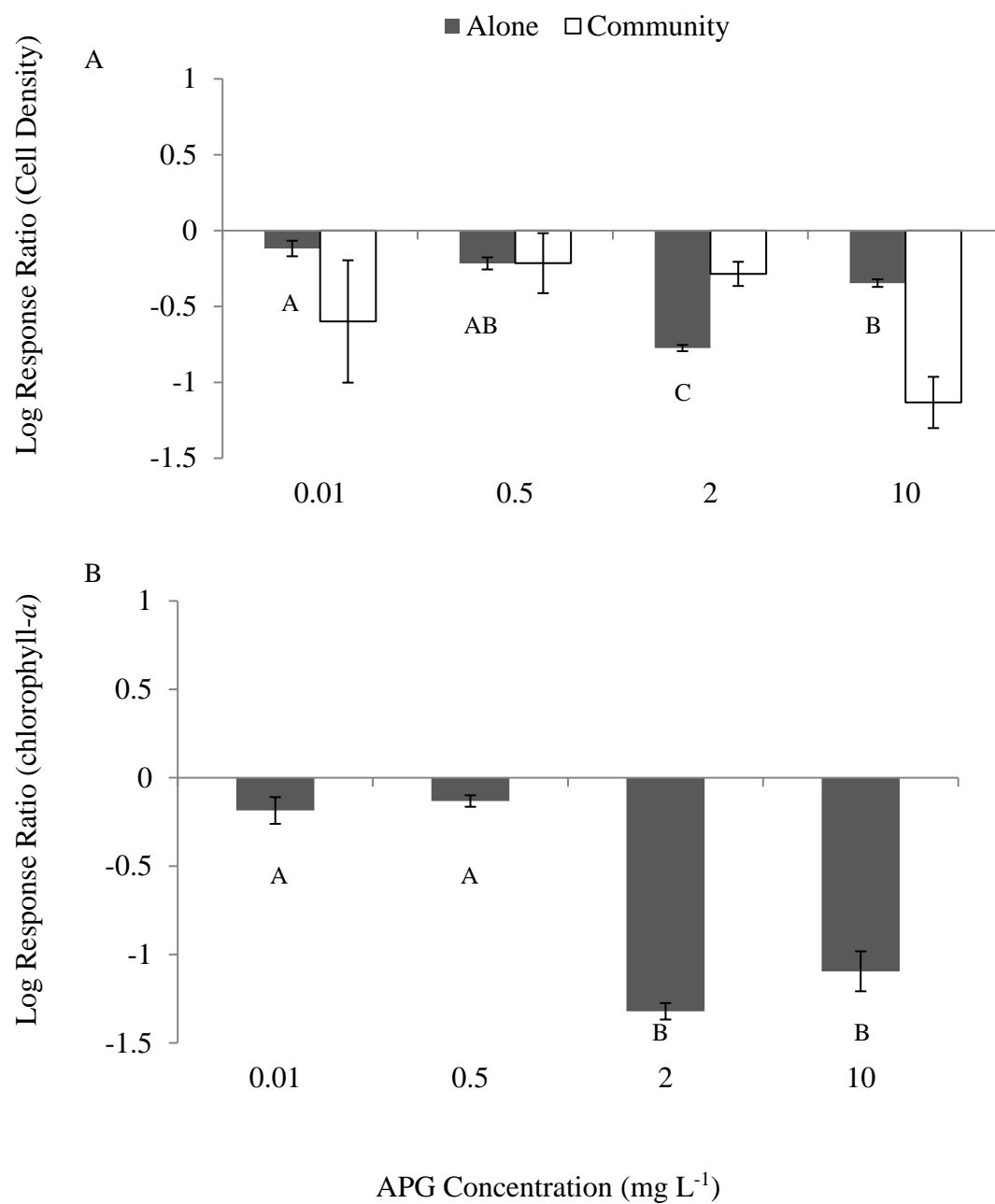


Figure 1.9. Mean LRR for *Navicula* sp. cell density in laboratory tests (n=6) alone and in community (A) and mean chlorophyll-a LRR (B). Letters represent Tukey HSD post-hoc comparisons. Treatment groups not connected by the same letter are significantly different from one another. Error bars are \pm one SEM.

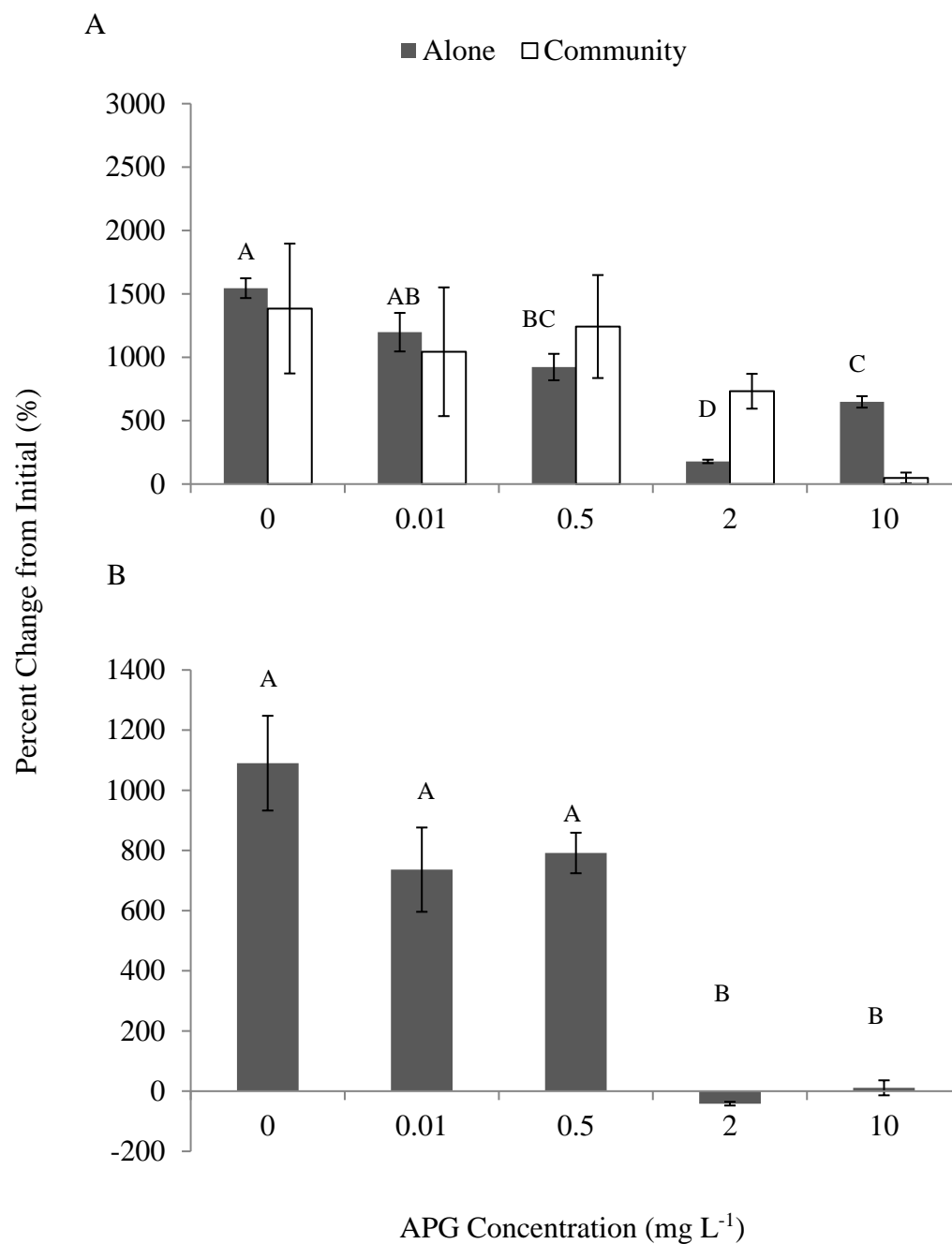


Figure 1.10. Mean percent change from initial cell density readings for *Navicula* sp. for cell density alone (n=6) and when in community (n=6)(A) and chlorophyll-*a* concentration for individual species test (B). Error bars are \pm one SEM.

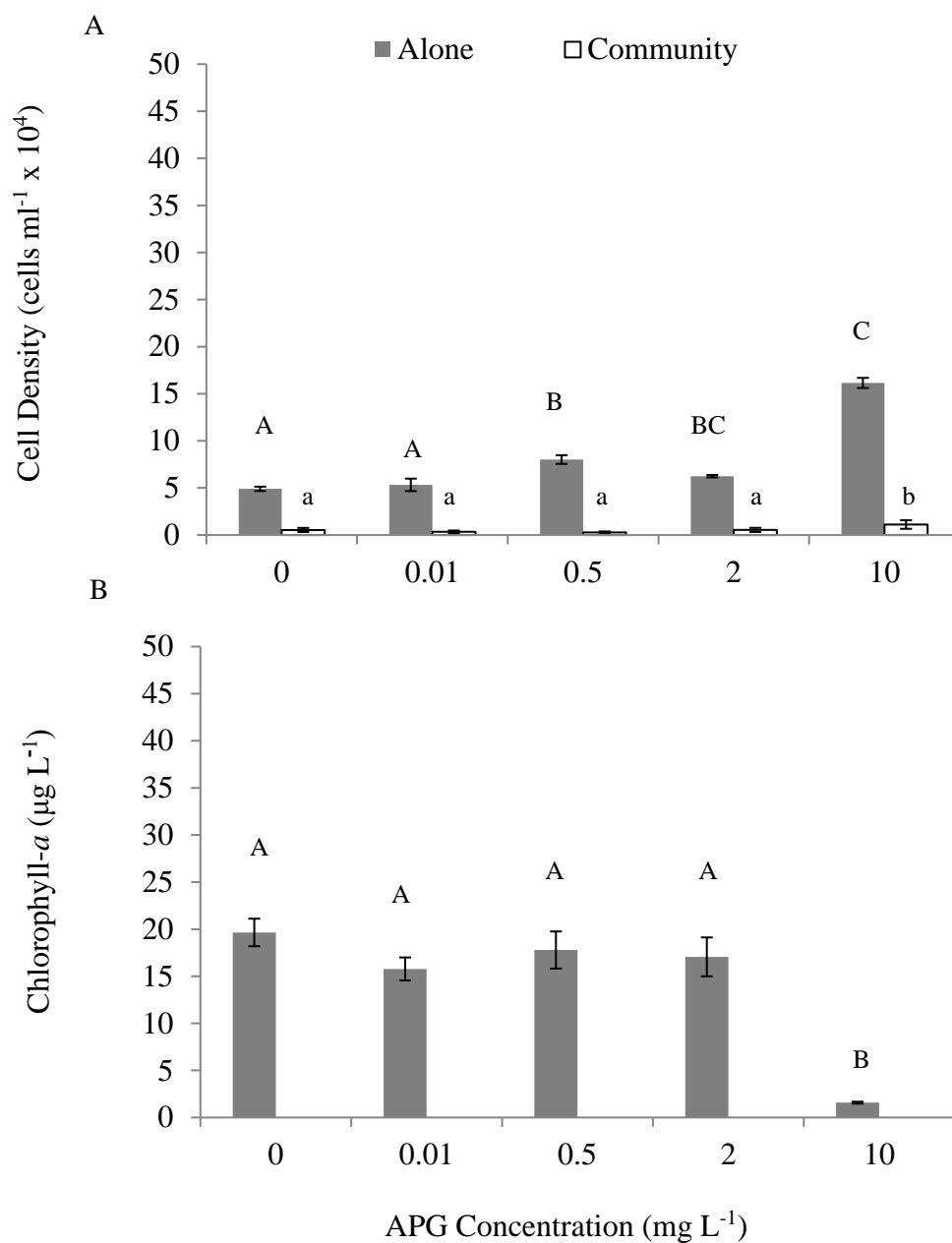


Figure 1.11. Mean cell densities of *Scenedesmus* sp. (A) and chlorophyll-*a* concentration (B) after seven days of exposure to 0, 0.01, 0.5, 2 and 10 mg L⁻¹ APG in individual species or community tests (n=6). Error bars are \pm one SEM. Comparisons for individual species are indicated by upper-case letters, while comparisons for the community are indicated by lowercase letters. Treatments not connected by the same letter are significantly different.

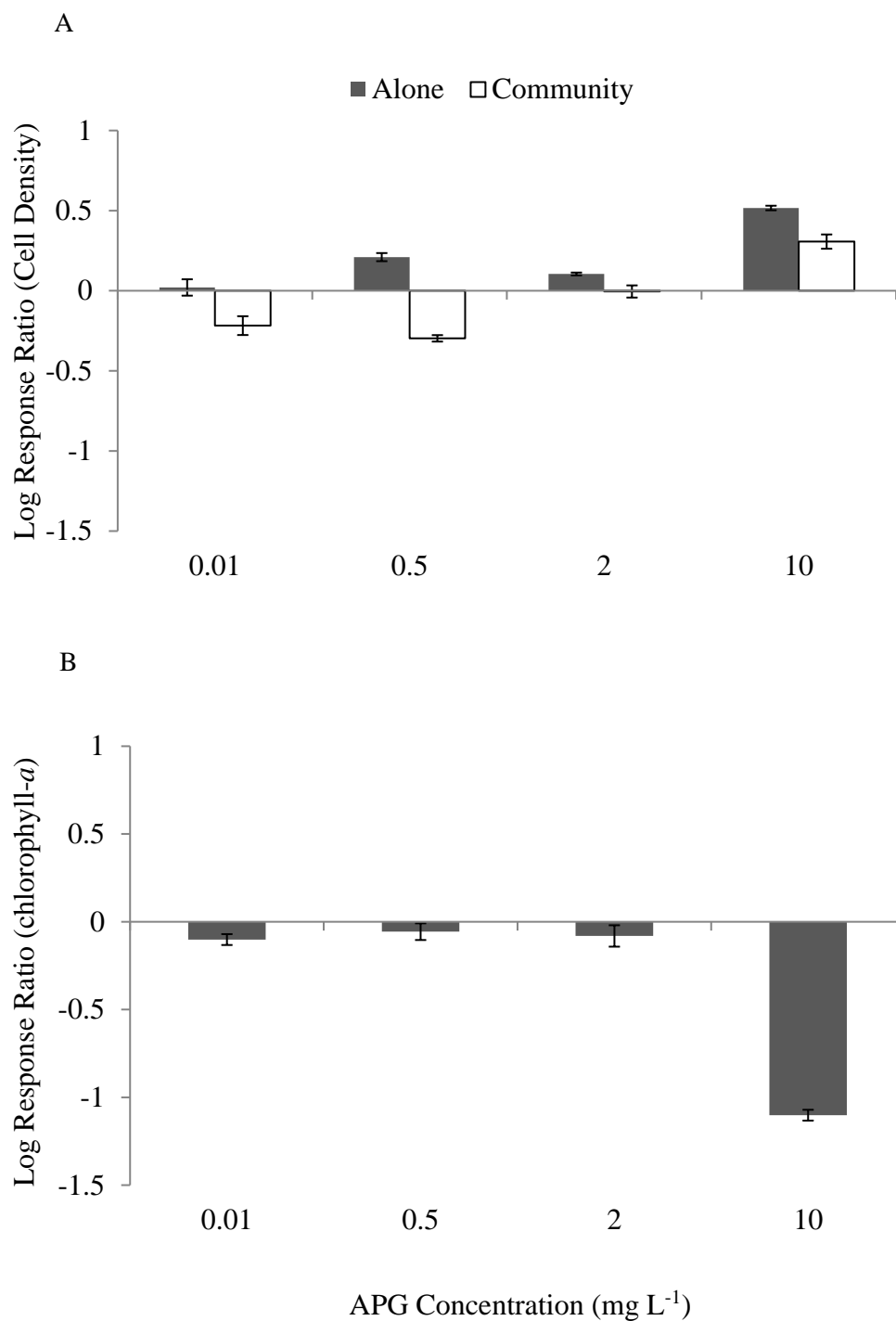


Figure 1.12. Mean LRR for *Scenedesmus* sp. cell density in laboratory tests (n=6) alone and in community (A) and mean chlorophyll-a LRR (B). Letters represent Tukey HSD post-hoc comparisons. Treatment groups not connected by the same letter are significantly different from one another. Error bars are \pm one SEM.

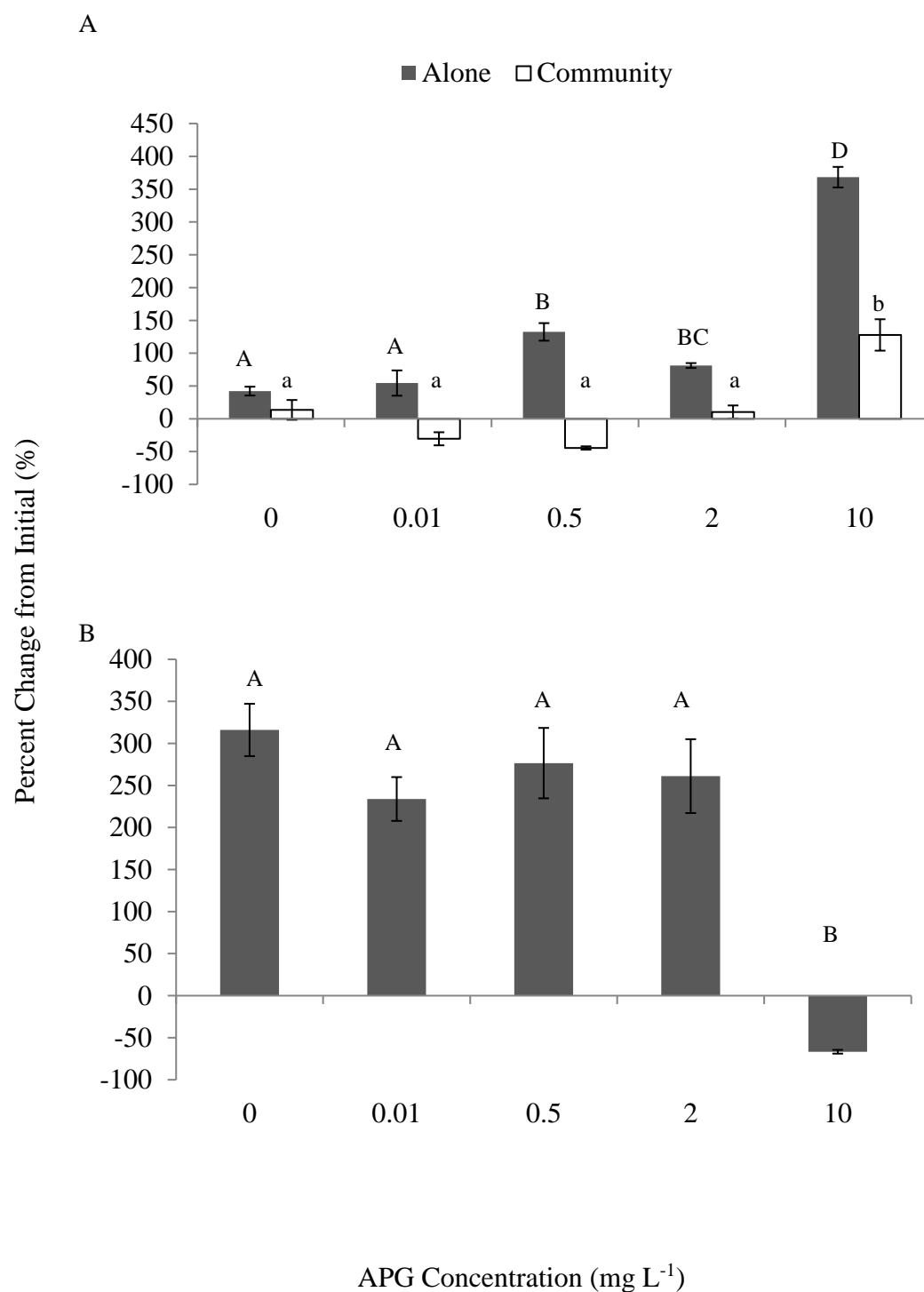


Figure 1.13. Mean percent change from initial cell density readings for *Scenedesmus* sp. for cell density alone (n=6) and when in community (n=6)(A) and chlorophyll-*a* concentration for individual species test (B). Error bars are \pm one SEM.

CHAPTER 2

FRESHWATER PHYTOPLANKTON COMMUNITY RESPONSES TO APG IN THE PRESENCE OR ABSENCE OF ZOOPLANKTON GRAZERS

INTRODUCTION

Food quality and availability for zooplankton grazers is largely determined by phytoplankton community composition. Phytoplankton communities are often composed of a variety of phytoplankton that vary in morphology, palatability, and nutritional quality (Porter and Orcutt 1980, Sterner et al. 1993, Brett and Muller-Navarra 1997, Choi et al. 2014). These factors ultimately determine whether phytoplankton taxa are preferred by grazers (Porter and Orcutt 1980, Brett and Muller-Navarra 1997). For example, fatty acids from phytoplankton were allocated selectively to enhance *Daphnia* growth and reproduction, while terrestrial particulate organic carbon inputs made only a minor contribution to *Daphnia* reproduction (Brett et al. 2009). While phytoplankton community composition influences energy transfer to zooplankton communities, zooplankton grazing can affect phytoplankton abundance. It is not uncommon for zooplankton to consume palatable phytoplankton at rates as fast, or faster, than the rates at which phytoplankton typically grow (Persson 1985, Børsheim and Anderson 1987). This grazing pressure along with changes in abiotic factors such as nutrient and light availability and temperature are major factors that contribute to phytoplankton community composition (Ryther and Sanders 1980, Malone and Neale 1981, Sommer et al. 1986, Sommer et al. 2012).

Disturbances such as pollution also alter grazing interactions (Pavlic et al. 2005, Carey et al. 2012). Freshwater ecosystems receive many types of chemical contaminants including surfactants. Surfactants are commonly used foaming agents and dispersants

used in personal care products and agricultural products (Bu et al. 2013). Surfactants enter aquatic systems following incomplete wastewater treatment and in surface runoff (Atkinson et al. 2009, Potter et al. 2014). One group of nonionic surfactants, alkyl polyglucosides (APG), was detected in surface waters in concentrations ranging from 0.013-0.017 mg L⁻¹ (Ghose et al. 2009, Traversa-Soto et al. 2015, EPA 2016), and is potentially damaging to phytoplankton communities (see Chapter 1).

There is some evidence that APG induces losses of grazers that can alter phytoplankton total abundance (Riera and Cohen 2016). However, it is unclear which phytoplankton taxa are affected most by APG, and how the presence or loss of grazers alters phytoplankton community responses to APG contamination. In the absence of zooplankton grazers exposure to 2 and 10 mg L⁻¹ APG reduced *Microcystis aeruginosa* cell densities in the lab and *Navicula* sp. cell densities in the field (see Chapter 1). Concentrations of 0.5, 2, and 10 mg L⁻¹ APG also decreased *Microcystis aeruginosa* cell densities by 45-70% when exposed in monoculture for one week under laboratory conditions (see Chapter 1). *Navicula* sp. cell densities were most affected by 2 and 10 mg L⁻¹ APG with decreases of 83% and 56% respectively (see Chapter 1). Furthermore, Payne and Hall (1977) found that the nonionic surfactant, C₁₄₋₁₅ alcohol ethoxylate, inhibited diatom and green algae growth at concentrations <100 mg L⁻¹. These studies indicate that APG influences phytoplankton abundance and that different taxa have different sensitivities to APG, however it is unclear how natural phytoplankton communities may respond in the presence or absence of zooplankton grazers.

Phytoplankton communities responses to surfactants may differ under natural temperature and light variation and in the presence of grazers. Which has important

implications for food quality or availability to grazers. In the presence of zooplankton grazers, overall phytoplankton abundance (chlorophyll *a* concentration) decreased by 80% after chronic exposure (≥ 7 days) to 2.5 mg L⁻¹ APG (Riera and Cohen 2016). While this study indicated that APG influences phytoplankton abundance, the role of grazing in community response to APG remains unclear. Phytoplankton communities of *Scenedesmus* sp. and *Chlorella vulgaris* responded to antibiotic treatments differently in the presence or absence of *D. magna*; without grazers, norfloxacin decreased colony size and abundance of *C. vulgaris*, while grazers caused norfloxacin to decrease colony size and dominance of *Scenedesmus* sp. (Pan et al. 2020). Testing phytoplankton responses to APG in the presence or absence of zooplankton grazers are needed to determine how aquatic communities respond to APG contamination.

Possible phytoplankton community responses to APG include direct and indirect effects. Duff et al. (2017) indicated the potential for direct effects of APG on *Chlorella* sp. abundance following exposure to 2 mg L⁻¹ APG. Chapter 1 also provides evidence for direct effects of APG on *Microcystis aeruginosa* and *Navicula* sp. It is also possible that APG could have density-mediated indirect effects on phytoplankton communities (Relyea and Hoverman 2006). In this case, density reduction of zooplankton grazers release phytoplankton from predation, influencing community composition. For example, zooplankton abundance and community composition were altered following one month of exposure to ≥ 2.5 mg L⁻¹ APG due mostly to losses of copepods, leaving communities dominated by cladocerans (Riera and Cohen 2016). Larger-bodied predatory zooplankton like copepods are important in mediating abundances of smaller zooplankton such as *D. magna* or *Bosmina* sp. (Byron et al. 1984, Santer 1993), which have the potential to cause

shifts in taxonomic composition of phytoplankton communities. It is also possible for a combination of direct chemical and indirect grazing effects (Pan et al. 2020). Thus, testing the toxicity of APG on phytoplankton in the presence or absence of zooplankton grazers is an important step in determining ecological effects of APG contamination.

While both phytoplankton and zooplankton have the potential to be influenced by APG, the effects of APG on the zooplankton/phytoplankton interaction has yet to be determined. The goal of this study was to compare ambient freshwater phytoplankton community composition following exposure to APG both in the presence and absence of zooplankton grazers in floating field microcosms. I hypothesized that APG concentration determines phytoplankton community composition, but greater losses of palatable taxa occurs in the presence of grazers due to consumption. It was also expected that largest reductions in phytoplankton abundance would occur in concentrations $\geq 2 \text{ mg L}^{-1}$.

METHODS

Experimental Design

Two field experiments were conducted using floating microcosms to assess the influence of APG on phytoplankton community composition in the presence and absence of zooplankton grazers. Experiments were conducted in a pond in Bulloch County, Georgia, USA (32°37'30" N, 81°52'30" W). The pond is approximately 9,000 m² in area, with approximately 3,700 m² at depths greater than 1.3 m (Riera and Cohen 2016). Microcosms were 20 L floating plastic containers (cubitainers)(Glibert et al. 2014). Microcosms were filled with 20 L of pond water using a 12v water pump (Delavan model 5850-201C, Minnesota, USA), keeping the intake hose 0.5 m below the surface to standardize filling. For the experiment without grazers, a 56 µm mesh was put over the intake hose to exclude most grazers. In filtered samples, no large-bodied zooplankton were counted in samples, but small rotifers were present in some of the samples. In the grazer-inclusion experiment, commonly observed taxa included were copepods, daphnids and *Bosmina*. Cubitainers were deployed and anchored with rope and stakes to the bottom of the pond in two parallel rows of 20 with 0.5 m of space between them. Microcosms were neutrally buoyant, therefore remaining submerged just under the surface of the pond. Containers were deployed approximately 20 m from the edge in order to avoid shading from nearby trees.

Establishment of APG Treatments

Freshwater pond phytoplankton communities were exposed to 0 (control), 0.01, 0.5, 2, or 10 mg L⁻¹ APG. Treatment solutions were mixed with Planteren 2000 (CAS:

68515-73-1, 110615-47-9), an APG compound typically used as a foaming agent and dispersant in personal care products (Bu et al. 2013), and ambient pond water. Concentrations selected include those measured in surface waters (0.01 and 0.5 mg L⁻¹; Ghose et al. 2009) and at a range that previously decreased total phytoplankton abundance (2 and 10 mg L⁻¹; Riera and Cohen 2016, Duff et al. 2017). Initial concentrations of 2 and 10 mg L⁻¹ were confirmed with the Anthrone Method using spectrophotometry (Buschmann and Wodarczak 1995, Schroder and Ventura 2000). This method is a colorimetric assay used to measure absorbance of samples. The anthrone reagent used in this analysis turns samples a yellow color, where samples with higher concentrations tend to be a darker color yellow (Buschmann and Wodarczak 1995, Schroder and Ventura 2000). For the experiment with phytoplankton without grazers, the average initial concentrations were 2.15 ± 0.39 and 10.36 ± 0.57 mg L⁻¹. For the experiment with grazers, the average concentrations initially were 2.63 ± 0.31 and 10.31 ± 0.44 mg L⁻¹. For both experiments, initial cell densities for communities within treatment groups were similar to one another (with grazers: Global R=0.119, Significance level=0.003, without grazers: Global R=0.063, Significance level=0.79). A power analysis was used to confirm n=4 would be adequate to detect differences in the communities (JMP Power Analysis, Power=0.81).

Sample Collection and Analysis

For both experiments, at both initial and final sampling times abiotic factors were measured first, then water samples were collected by first shaking cubitainers to homogenize the phytoplankton community before collecting 200 ml subsamples. Samples

were collected from each container initially and after one week of exposure to treatments. The 200 ml samples were transported back to the lab in a dark cooler. Once in the lab, samples were subdivided into aliquots for phytoplankton identification (25 ml) and chlorophyll *a* analysis (100 ml). The 25 ml sample for phytoplankton identification was preserved using gluteraldehyde for subsequent identification to the lowest taxonomic level using an EVOS FL compound microscope and 400X total magnification (Erdogan and Yerli 2014). The sample was shaken to homogenize before 1 ml of sample was pipetted into a Sedgewick-Rafter chamber and allowed to settle for at least one hour before analysis. Samples were then counted in transects to count one half of the cells in the chamber and used to calculate cells ml⁻¹ (Erdogan and Yerli 2014). *Microcystis* sp. was counted as colonies (Table 2.1). Since *Microcystis* sp. cells were not counted individually they were excluded from statistical analyses.

Chlorophyll a Concentration

Chlorophyll *a* (n=8) was also used as a proxy for total phytoplankton abundance. Water samples (100 ml) were filtered through glass microfiber filters (Whatman GF/F; nominal pore size 0.7 µm) to collect phytoplankton cells. Pigments from collected phytoplankton were then extracted in 90% acetone in the dark at -20°C for 24 hours followed by analysis on a Trilogy Fluorometer (Turner Designs, CA, USA) according to EPA protocol 445.0 (Arar and Collins 1997).

Statistical Analysis

Differences in chlorophyll *a* concentration across APG treatments were analyzed using one-way ANOVA followed by pairwise Tukey HSD post-hoc comparisons. Prior to analysis, all data were tested for normality using Shapiro-Wilk *W* tests, and homogeneity of variances using Levene's test. All chlorophyll *a* analyses were performed using JMP Pro 13 (SAS Institute Inc., Cary, NC, USA). JMP was also used to perform t-tests for differences in abiotic factors at the beginning of the two experiments. Measurements for abiotic factors and t-test results show no differences in starting conditions for the two experiments (Table 2.2A,B).

Changes in the composition of the phytoplankton community in response to APG concentration were determined by using Bray-Curtis resemblance matrices followed by two-way repeated measures permutational multivariate analysis of variance (PERMANOVA+ add on; PRIMER-E v.7, Plymouth Marine Laboratory, U.K.)(Clarke and Gorley 2006). Before creating resemblance matrices, data were square root transformed to down-weight contributions of dominant species (Clarke and Gorley 2006). A dummy species pre-treatment was also added to compensate for assemblages that would otherwise result in undefined Bray-Curtis coefficients due to dividing by zero (Clarke and Gorley 2006). The PERMANOVA was then used as a non-parametric multivariate test with APG treatment and time as factors while accounting for the repeated measures design. After significant results, PERMANOVA pairwise comparisons were performed using ANOSIM and SIMPER as post-hoc tests to determine which taxa were contributing to differences in treatment groups. Data were first square-root transformed and then a Bray-Curtis resemblance matrix was calculated and used for the

ANOSIM. The contributions of each taxon to dissimilarities between treatment groups was then determined using PRIMER's similarity percentages (SIMPER) routine.

To account for differences from conducting experiments at different times *Chlorella* sp. cell density data were converted to log response ratios (LRR). LRR is used in ecological analyses to summarize results of experiments or compare results in meta-analyses (Lajeunesse 2015). The LRR formula used was $LRR = \log_{10}(\text{final cell density} / \text{initial cell density})$. Chlorophyll-*a* data were also compared using LRR. LRRs for each experiment were then tested for differences using ANOVA in JMP.

RESULTS

Phytoplankton community with zooplankton grazers

Phytoplankton community composition in the presence of grazers was influenced by APG treatment (PERMANOVA, Psuedo-F = 3.40, $p = 0.0001$; ANOSIM, Global R = 0.12, Significance level = 0.0003) (Table 2.3,2.4A) and time (PERMANOVA, Psuedo-F = 27.14, $p = 0.0001$) (Table 2.3). Communities in the 2 and 10 mg L⁻¹ treatments differed from the control (Figure 2.1) (Table 2.4B). *Chlorella* sp. was the main contributor to the dissimilarities for all treatment groups. For differences in the communities in the 0 and 2 mg L⁻¹ treatments *Chlorella* sp. contributed 23% to dissimilarities, while *Navicula* sp. and *Tabellaria* sp. contributed 11% (Table 2.5; Figure 2.2,2.3). Differences between the 0 and 10 mg L⁻¹ treatments were also due to reductions in *Chlorella* sp. (21%), *Navicula* sp. (13%), and *Tabellaria* sp. (9%)(Table 2.5; Figure 2.2,2.3). The 0.01 and 10 mg L⁻¹ treatments also differed due to reductions in *Chlorella* sp. (27%) and *Navicula* sp. (10%), while *Peridinium* sp. accounted for 9.3% of dissimilarity (Table 2.5; Figure 2.2,2.3). Reductions in *Chlorella* sp. compared to control can be visualized in Figure 2.2. For the 0.5 and 10 mg L⁻¹ treatments, differences in communities were due to reductions of *Chlorella* sp. (20%), *Anabaena* sp. (10%), and *Navicula* sp. (8%). Differences between the 2 and 10 mg L⁻¹ treatment were due to reductions of *Chlorella* sp. (29%), *Tabellaria* sp. (9%), and *Peridinium* sp. (9%)(Table 2.5, Figure 2.2,2.3). Overall, green algae and diatoms such as *Chlorella* sp., *Navicula* sp., and *Tabellaria* sp. accounted for dissimilarities. Though cyanobacteria (*Anabaena* sp.) and dinoflagellates (*Peridinium* sp.) also accounted for dissimilarities in some treatments.

Chlorella sp. LRRs for cell density show that in the presence of grazers APG did influence *Chlorella* sp. response (ANOVA, $F_{4,19} = 3.82$, $p = 0.02$) (Figure 2.4). In the presence of grazers concentrations $\geq 0.5 \text{ mg L}^{-1}$ APG had similar effects on *Chlorella* sp. LRR (ANOVA, $F_{4,39} = 5.27$, $p = 0.002$) (Figure 2.4). In contrast, LRR for chlorophyll-*a* concentration was only different from control in the 10 mg L^{-1} concentration (ANOVA, $F_{4,39} = 8.85$, $p = <0.0001$) (Figure 2.5).

Phytoplankton community without zooplankton grazers

In the absence of zooplankton grazers, APG did not influence community composition but the community changed over time (Table 2.6; Figure 2.6). Furthermore, *Chlorella* sp. LRR was only different from control in the 10 mg L^{-1} treatment (Figure 2.4). Chlorophyll-*a* concentration LRR followed a similar pattern, with the only response different from controls coming in the 10 mg L^{-1} treatment (ANOVA, $F_{4,39} = 7.67$, $p = 0.0002$) (Figure 2.5). The 10 mg L^{-1} treatment without grazers was the only treatment to have a negative chlorophyll-*a* response.

DISCUSSION

Results from this study indicate that APG effects on phytoplankton community composition are stronger in the presence of zooplankton grazers. The hypothesis was only supported when zooplankton grazers were present, indicating that grazing influences the toxicity of APG. It was expected that APG would effect phytoplankton community composition with and without grazers which did not happen. We also expected that most severe effects with grazers would be on palatable taxa. In the presence of grazers *Chlorella* sp. (green algae) and *Navicula* sp. (diatom) were responsible for dissimilarities among many of the treatment groups. This result was not expected because zooplankton typically prefer to consume green algae and diatoms compared to less palatable and lower quality food sources such as cyanobacteria or dinoflagellates like *Peridinium* sp. (Porter and Orcutt 1980, Sterner et al. 1993, Brett and Muller-Navarra 1997, Choi et al. 2014). These findings support the prediction that the abundance of palatable taxa would be reduced more by APG in the presence of zooplankton grazers. The reduction of palatable taxa is likely explained by reduced grazing pressure in the absence of grazers. Grazing can significantly influence phytoplankton growth because some grazers are able to consume phytoplankton at rates faster than the rates phytoplankton can grow (Persson 1985, Børsheim and Anderson 1987). The absence of grazers likely allowed for palatable and high quality food taxa such as *Chlorella* sp. growth to remain similar among APG treatments less than 10 mg L⁻¹.

It was predicted that APG effects on phytoplankton would occur in concentrations ≥ 2 mg L⁻¹ APG. However, *Chlorella* sp. LRR did differ from control in the 0.5 mg L⁻¹ treatment. These results somewhat agree with previous studies that concluded APG

effects on phytoplankton are greatest at concentrations $\geq 2 \text{ mg L}^{-1}$ (Riera and Cohen 2016, Duff et al. 2017, see Chapter 1), but also suggest that environmentally relevant concentrations like 0.5 mg L^{-1} may influence phytoplankton growth in the presence of grazers. Chlorophyll-*a* LRR also generally support this prediction since only the 10 mg L^{-1} treatment differed from control.

Reductions in cell abundance were due mostly to reductions of cells preferred by grazers (*Chlorella* sp. and *Navicula* sp.). Reductions of taxa that represent high quality food could affect energy transfer to zooplankton, ultimately influencing the abundance or reproduction of grazers (McQueen et al. 1986, Persson et al. 2008, Choi et al. 2016). Most reductions were due to *Chlorella* sp., which is a species of phytoplankton found in many different freshwater systems. Since *Chlorella* sp. reductions occurred during months when green algae densities are already low, it is possible that food availability to grazers was reduced (Sommer et al. 1986, Fonseca and Bicudo 2008). However, reductions in abundance for other taxa also occurred in most APG concentrations, suggesting that *Chlorella* sp. and *Navicula* sp. are not the only taxa sensitive to APG treatment. This suggests that in other types of freshwater systems with different phytoplankton communities that a wide variety of taxa may be influenced by APG.

Influences on phytoplankton community composition also occurred at concentrations of 0.01 and 0.5 mg L^{-1} , which are considered to be environmentally relevant (Ghose et al. 2009, Traversa-Soto et al. 2015). This suggests that even low concentrations of APG can influence freshwater food webs. Future studies on phytoplankton communities should focus on exposing phytoplankton for longer time periods. Doing longer experiments may better show the influence that APG has on phytoplankton growth and production and how it may affect

food availability to higher trophic levels. It is also important to consider how APG toxicity may be influenced by temperature as global temperatures increase since high temperatures favor the growth of cyanobacteria compared to other species (Ryther and Sanders 1980, Malone and Neale 1981, Sommer et al. 1986, Sommer et al. 2012).

Warmer water temperatures tend to favor the growth of cyanobacteria such as *Microcystis* sp. If densities of palatable taxa are low to begin with, reductions in abundance due to APG could further reduce food availability to grazers and ultimately higher trophic levels.

Table 2.1. Microcystis counts \pm SEM with and without grazers following seven days of APG exposure.

APG Concentration	Grazers Colonies ml⁻¹	No Grazers Colonies ml⁻¹
0	133 \pm 6.5	116 \pm 8.7
0.01	138 \pm 5.3	120 \pm 5.1
0.5	125 \pm 16.7	127 \pm 10.1
2	121 \pm 6.5	119 \pm 8.8
10	120 \pm 5.1	125 \pm 11.4

Table 2.2. Mean initial measurements of water quality parameters between the two experiments (A) \pm SEM and t-tests for differences in parameters (B). Significant differences are noted with an asterisks.

A

Abiotic Factor	Experiment with Grazers	Experiment without Grazers
Temperature	31.0 ± 0.08	30.8 ± 0.19
pH	5.8 ± 0.02	6.4 ± 0.02
DO	6.5 ± 0.06	7.5 ± 0.04
EC	20.2 ± 0.56	23.7 ± 1.73

B

Abiotic Factor	t Ratio	DF	Prob < t
Temperature	-1.96	54.4	0.0275*
pH	35.0	77.9	1.00
DO	32.0	70.7	1.00
EC	4.29	47.0	1.00

Table 2.3. PERMANOVA results for comparisons of phytoplankton community composition responses to APG treatment after seven days of exposure with zooplankton grazers. Significant differences are noted with an asterisks.

Source	df (Effect, Total)	Pseudo-F	<i>p</i>
Treatment	4,79	3.40	0.0001*
Time	1,79	27.14	0.0001*
Cubitainer(Treatment)	35,79	0.83	0.9111
Treatment*Time	4,79	1.59	0.0587

Table 2.4. Pairwise tests for differences in community composition between treatments for the experiment with zooplankton grazers.

Groups	R statistic	Significance Level
0, 0.01	0.047	0.168
0, 0.5	0.079	0.109
0, 2	0.121	0.043*
0, 10	0.02	0.0002*
0.01, 0.5	-0.026	0.661
0.01, 2	0.017	0.349
0.01, 10	0.29	0.0003*
0.5, 2	-0.012	0.579
0.5, 10	0.199	0.0009*
2, 10	0.311	0.0006*

Table 2.5. SIMPER results displaying mean dissimilarity between treatments and the taxa that were most responsible for dissimilarities between treatment groups. Table includes the three taxa for each treatment with highest percent contributions for dissimilarities between groups.

Treatment Groups	Mean Dissimilarity	Species	Percent Contribution	Cumulative Percent
0 & 0.01	14.56	<i>Chlorella</i> sp.	23.6	23.6
		<i>Tabellaria</i> sp.	9.0	32.68
		<i>Navicula</i> sp.	8.9	41.59
0 & 0.5	16.61	<i>Chlorella</i> sp.	19.3	19.3
		<i>Tabellaria</i> sp.	9.6	29.0
		<i>Anabaena</i> sp.	9.2	38.2
0 & 2	16.53	<i>Chlorella</i> sp.	20.4	20.4
		<i>Navicula</i> sp.	11.8	32.3
		<i>Tabellaria</i> sp.	11.2	43.5
0 & 10	15.8	<i>Chlorella</i> sp.	21.2	21.2
		<i>Navicula</i> sp.	13.8	35.0
		<i>Tabellaria</i> sp.	9.0	44.1
0.01 & 0.5	13.7	<i>Chlorella</i> sp.	20.5	20.5
		<i>Anabaena</i> sp.	11.8	32.4
		<i>Eutonia</i> sp.	7.5	39.9
0.01 & 2	13.5	<i>Chlorella</i> sp.	20.6	20.6
		<i>Navicula</i> sp.	9.2	29.8
		<i>Peridinium</i> sp.	9.0	38.8
0.01 & 10	13.7	<i>Chlorella</i> sp.	27.9	27.9
		<i>Navicula</i> sp.	10.6	38.6
		<i>Peridinium</i> sp.	9.3	47.9
0.5 & 2	14.9	<i>Chlorella</i> sp.	20.0	20.0
		<i>Anabaena</i> sp.	11.1	31.1
		<i>Eutonia</i> sp.	8.2	39.4
0.5 & 10	15.0	<i>Chlorella</i> sp.	20.0	20.0
		<i>Anabaena</i> sp.	10.4	30.5
		<i>Navicula</i> sp.	8.3	38.8
2 & 10	14.9	<i>Chlorella</i> sp.	29.5	29.5
		<i>Tabellaria</i> sp.	9.7	39.2
		<i>Peridinium</i> sp.	9.3	48.5

Table 2.6. PERMANOVA results for comparisons of phytoplankton community composition responses to APG treatment after seven days of exposure without zooplankton grazers. Significant differences noted with an asterisks.

Source	df (Effect, Total)	Pseudo-F	<i>p</i>
Treatment	4,39	0.91	0.57
Time	1,39	48.43	0.0001*
Cubitainer(Treatment)	15,39	0.80	0.81
Treatment*Time	4,39	0.95	0.52

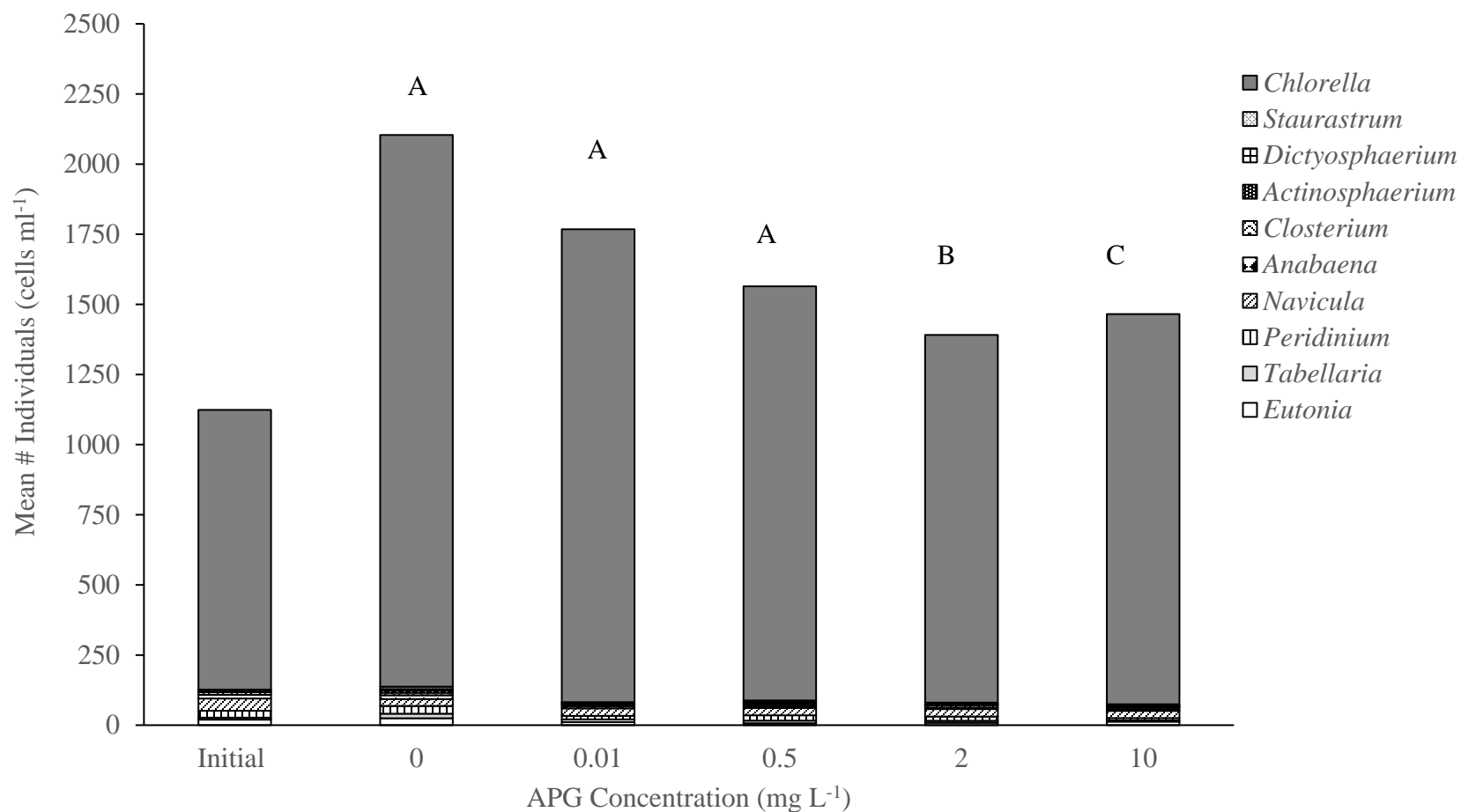


Figure 2.1. Mean total abundance of phytoplankton of each species initially and after seven days of exposure to 0, 0.01, 0.5, 2 or 10 mg L⁻¹ APG in the presence of zooplankton grazers (n=8). Significant differences in community composition are indicated by different letters

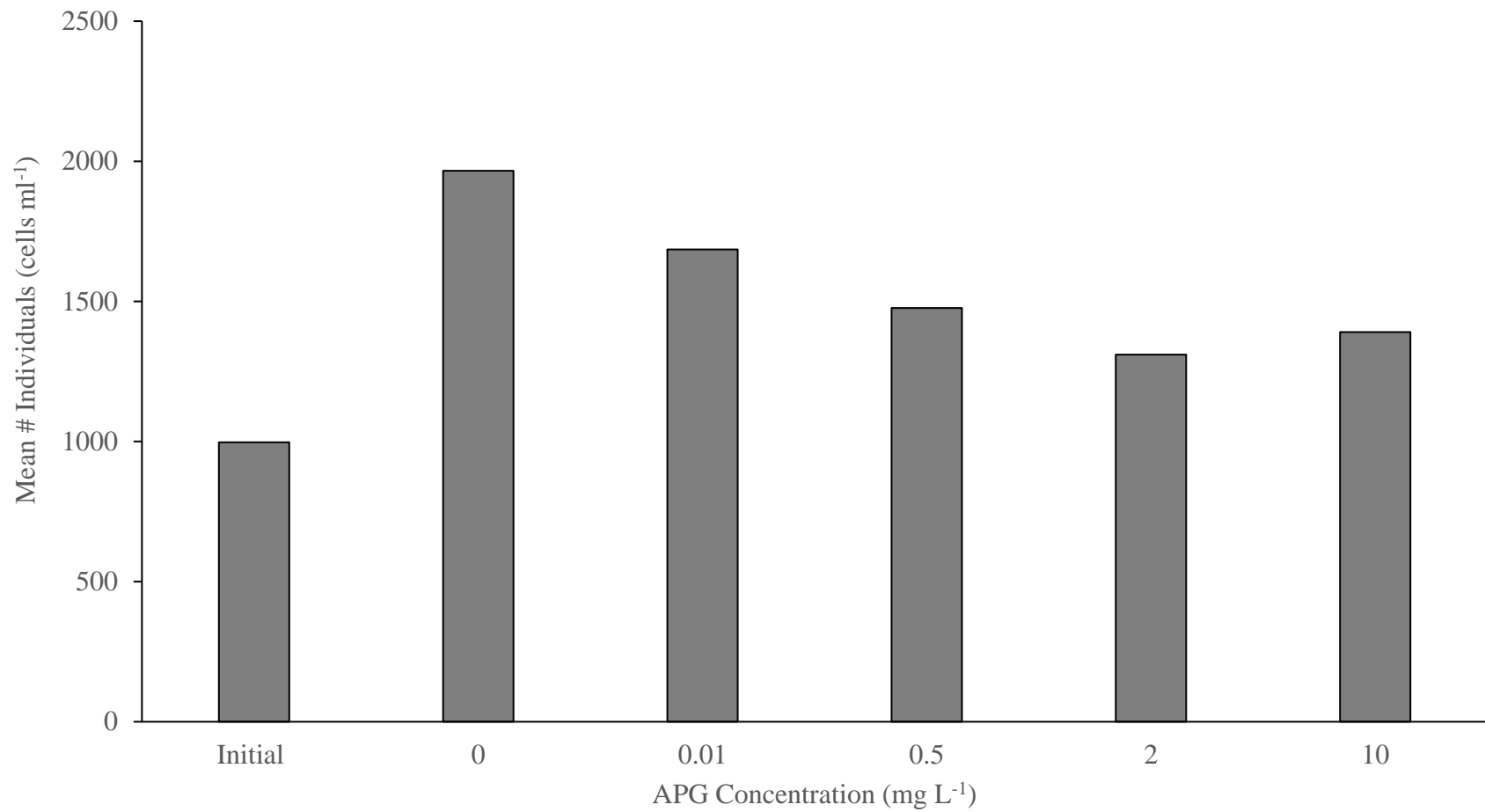


Figure 2.2. Mean total abundance of *Chlorella* sp. initially and after seven days of exposure to 0, 0.01, 0.5, 2 or 10 mg L⁻¹ APG in the presence of zooplankton grazers (n=8).

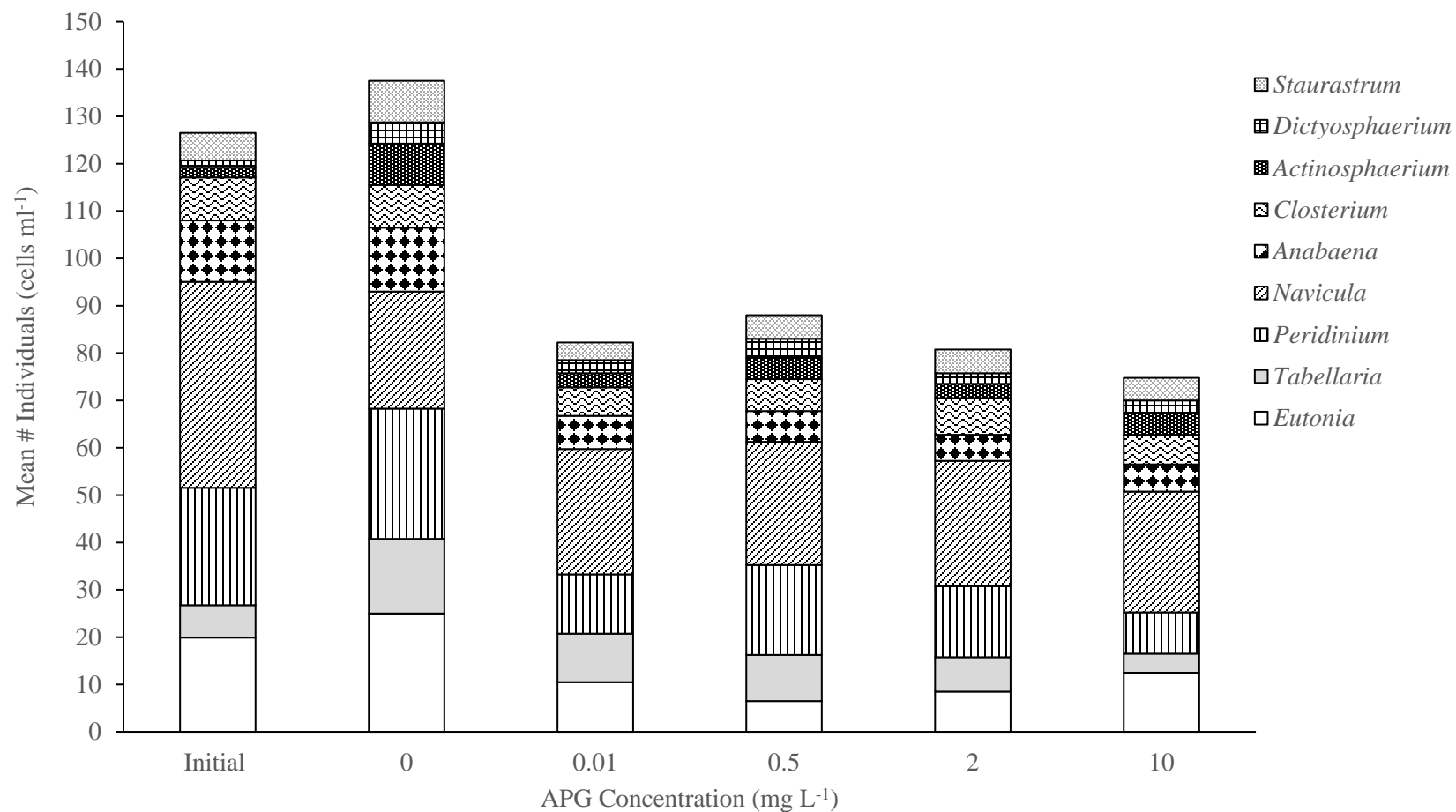


Figure 2.3. Mean total abundance of all other species initially and after seven days of exposure to 0, 0.01, 0.5, 2 or 10 mg L⁻¹ APG in the presence of zooplankton grazers (n=8).

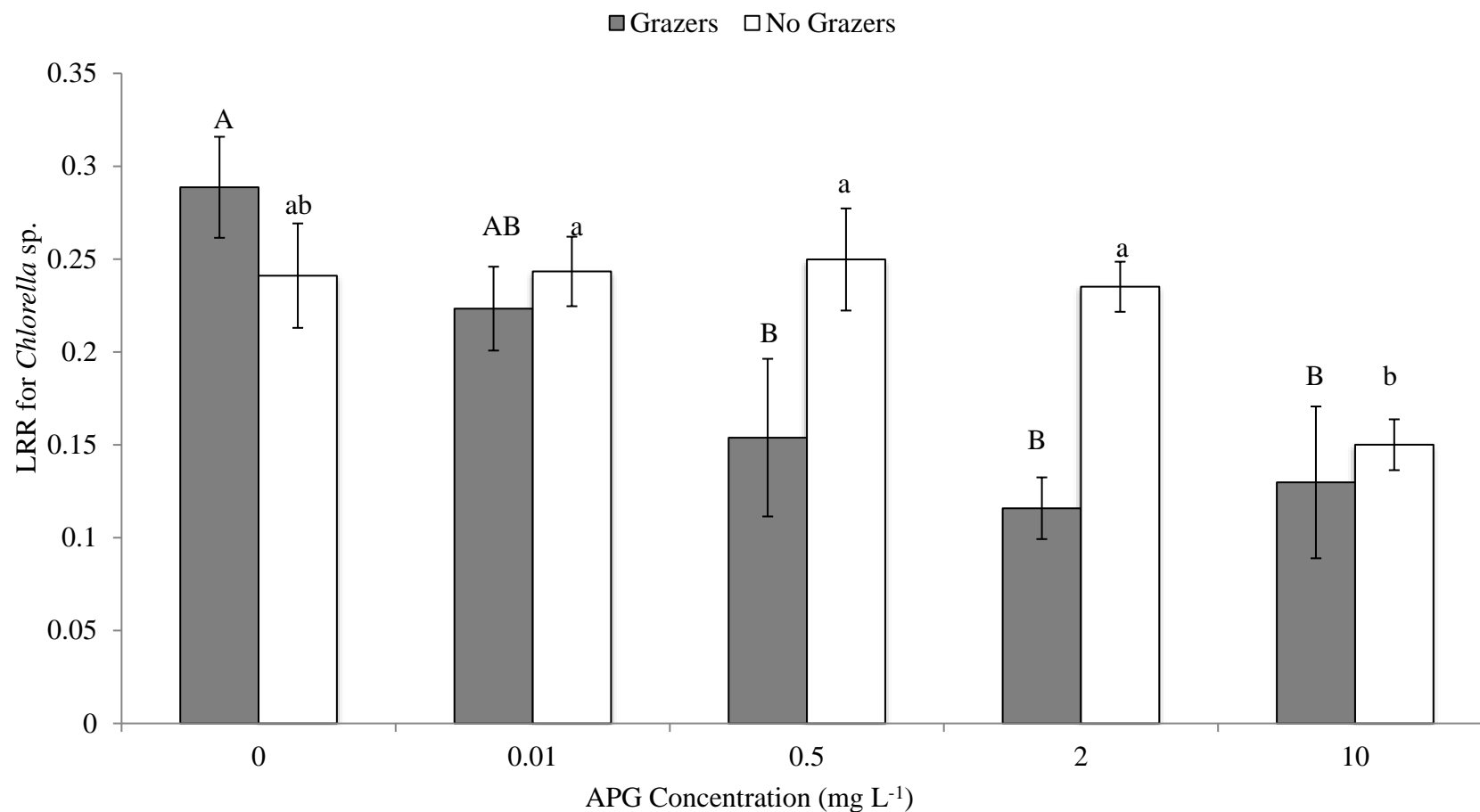


Figure 2.4. Log Response Ratios for *Chlorella* sp. after seven days of exposure to 0, 0.01, 0.5, 2 or 10 mg L⁻¹ APG in the presence or absence of zooplankton grazers. Significant differences in LRR are indicated by uppercase letters for grazers and lowercase for no grazers. Error bars are \pm SEM.

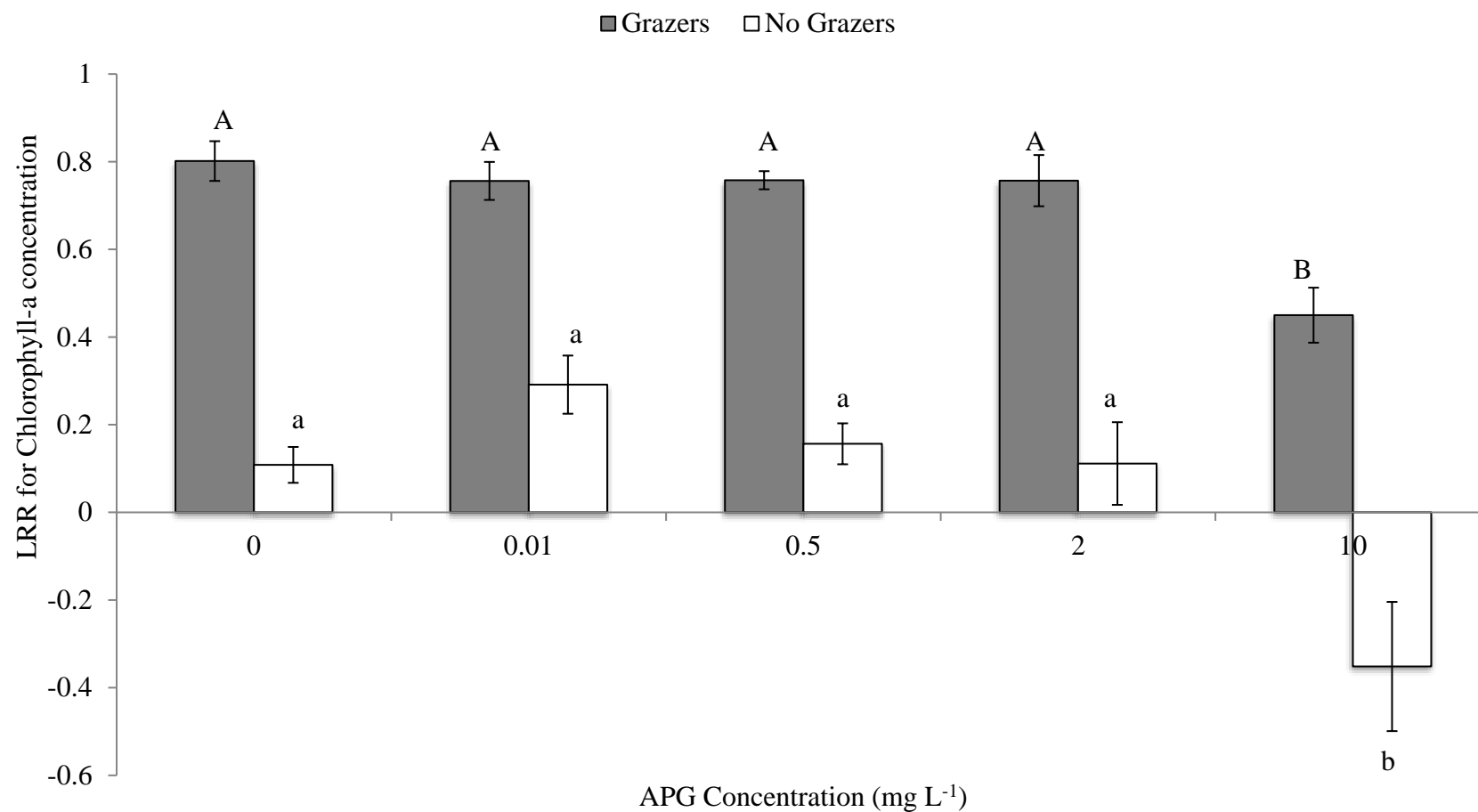


Figure 2.5. Log Response Ratios for chlorophyll-*a* concentration after seven days of exposure to 0, 0.01, 0.5, 2 or 10 mg L⁻¹ APG in the presence or absence of zooplankton grazers. Significant differences in LRR are indicated by uppercase letters for grazers and lowercase for no grazers.

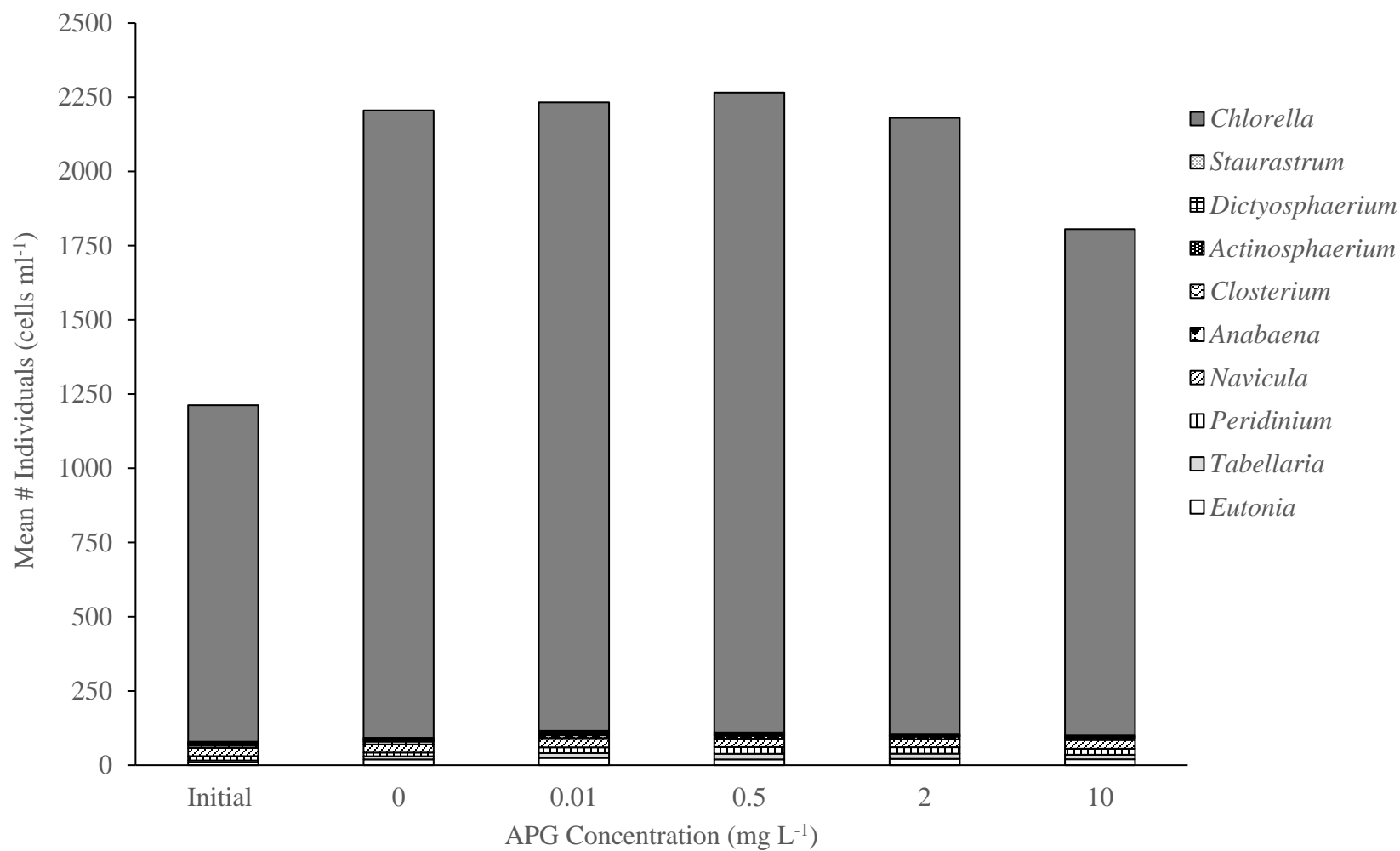


Figure 2.6. Mean total abundance of phytoplankton of each species initially and after seven days of exposure to 0, 0.01, 0.5, 2 or 10 mg L⁻¹ APG in the absence of zooplankton grazers (n=4).

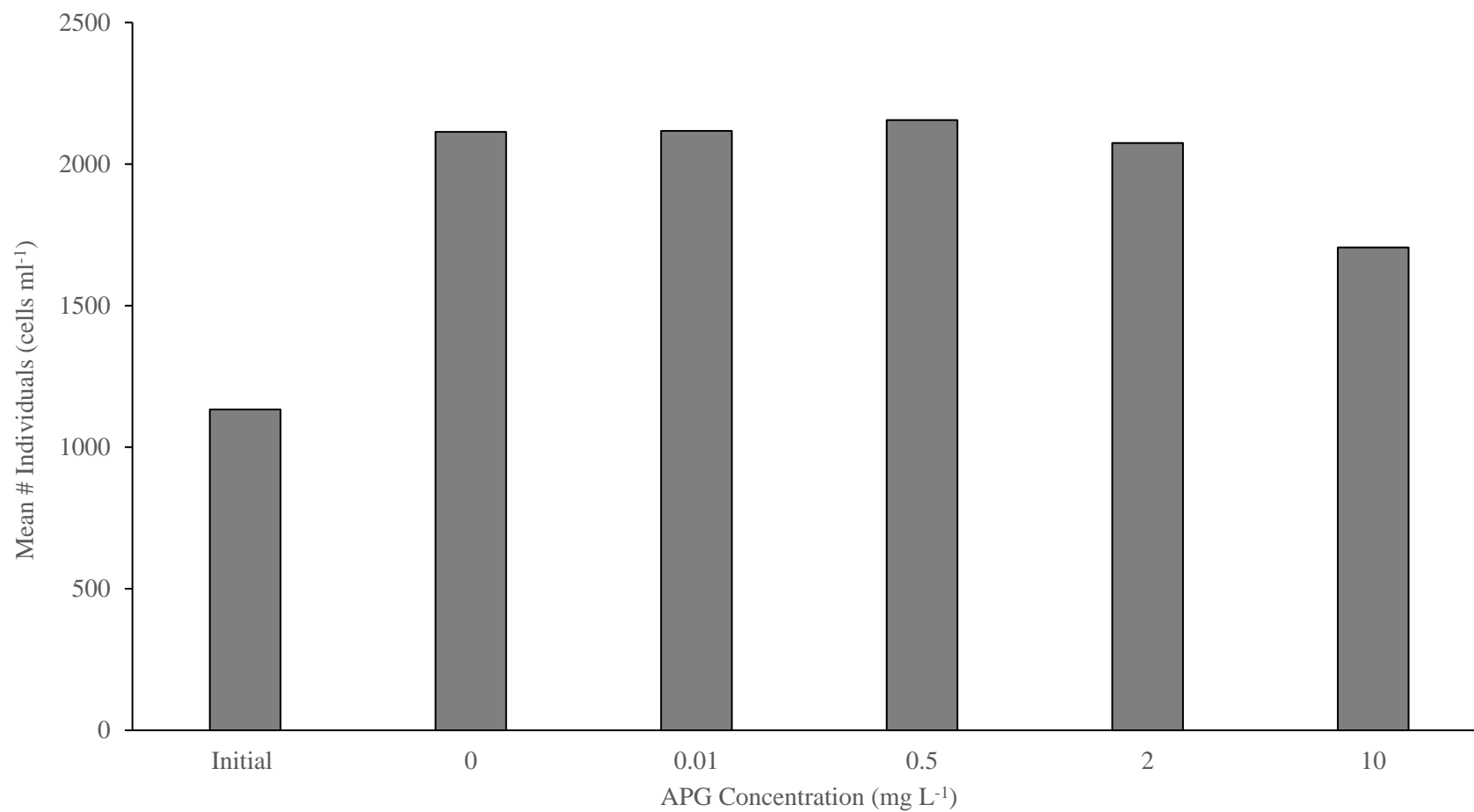


Figure 2.7. Mean total abundance of *Chlorella* sp. initially and after seven days of exposure to 0, 0.01, 0.5, 2 or 10 mg L⁻¹ APG in the absence of zooplankton grazers (n=4).

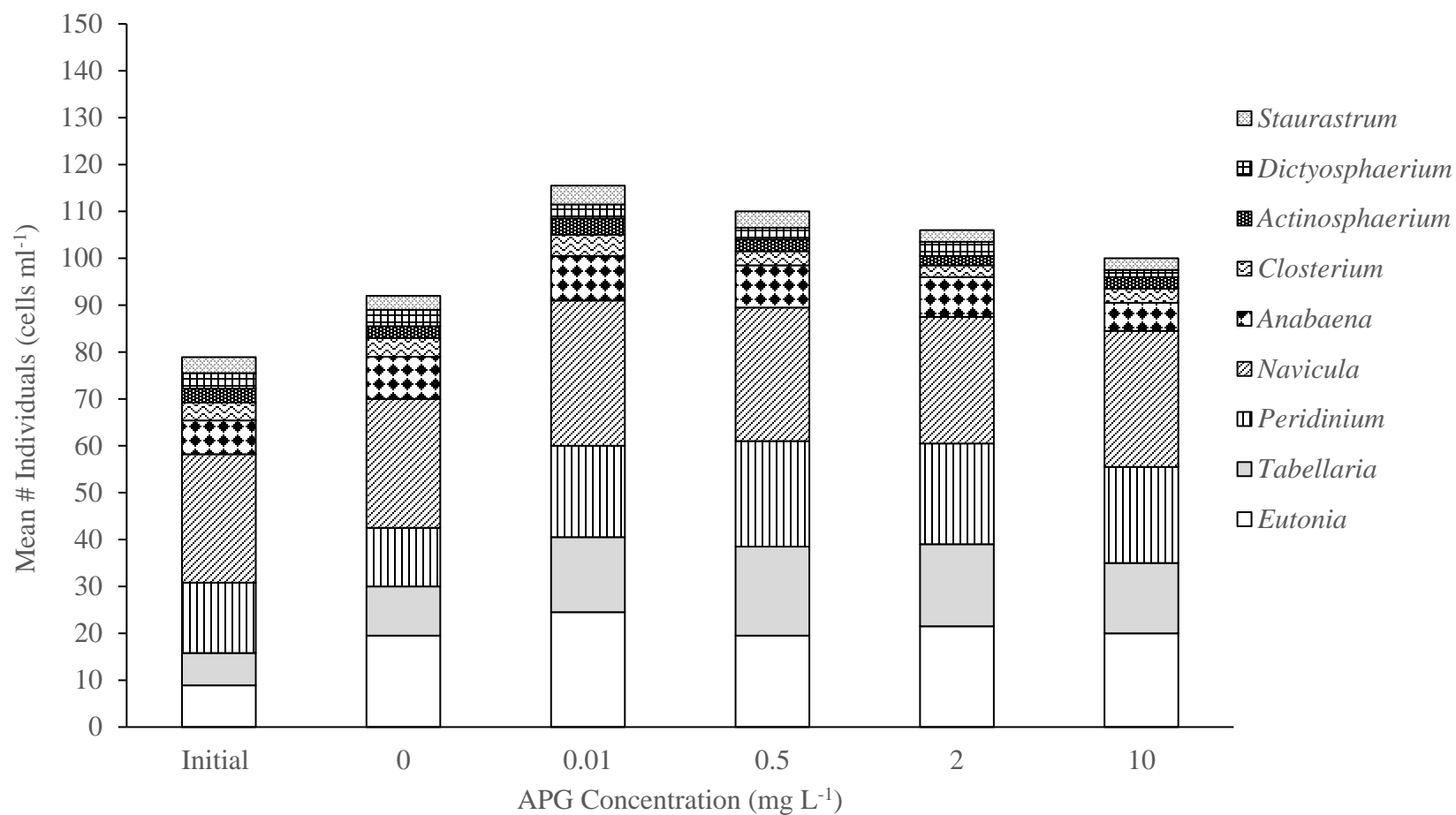


Figure 2.8. Mean total abundance of all other species initially and after seven days of exposure to 0, 0.01, 0.5, 2 or 10 mg L⁻¹ APG in the absence of zooplankton grazers (n=4).

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